Rooke, A.
10/506903


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     (FILE 'REGISTRY' ENTERED AT 14:22:38 ON 08 APR 2005)
                E CALCIUM
                E CALCIUM/CN 5
L1            1 S E3
                E CALCIUM IONS/CN 5
                E CALCIUM ION/CN 5
L2            1 S E3
L3            2 S L1 OR L2
L4           55 S "A-LACTALBUMIN"?/CN

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L4           55 SEA FILE=REGISTRY ABB=ON  PLU=ON  "A-LACTALBUMIN"?/CN

L5         6619 SEA FILE=CAPLUS ABB=ON  PLU=ON  L4 OR LACTALBUMIN
L6          574 SEA FILE=CAPLUS ABB=ON  PLU=ON  L5 AND (L3 OR (CA OR
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L7           24 SEA FILE=CAPLUS ABB=ON  PLU=ON  L6 AND (MUTAT? OR MUTAGEN?
                OR POLYMORPH? OR POLY MORPH? OR MUTANT)


L1            1 SEA FILE=REGISTRY ABB=ON  PLU=ON  CALCIUM/CN
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L3            2 SEA FILE=REGISTRY ABB=ON  PLU=ON  L1 OR L2
L4           55 SEA FILE=REGISTRY ABB=ON  PLU=ON  "A-LACTALBUMIN"?/CN

L5         6619 SEA FILE=CAPLUS ABB=ON  PLU=ON  L4 OR LACTALBUMIN
L6          574 SEA FILE=CAPLUS ABB=ON  PLU=ON  L5 AND (L3 OR (CA OR
                "CA+")(S)CALCIUM OR CALCIUM)
L8            3 SEA FILE=CAPLUS ABB=ON  PLU=ON  L6 AND (K79 OR D82 OR D84
                OR D87# OR D88 OR S70R)

L9           24 L7 OR L8

L9   ANSWER 1 OF 24  CAPLUS  COPYRIGHT 2005 ACS on STN
ED   Entered STN:  07 May 2004
ACCESSION NUMBER:         2004:371064  CAPLUS
DOCUMENT NUMBER:          140:373461
TITLE:                    Evaluation of breast cancer states and outcomes
                          using gene expression profiles
INVENTOR(S):              West, Mike; Nevins, Joseph R.; Huang, Andrew
PATENT ASSIGNEE(S):       Synpac, Inc., USA; Duke Univerisity
SOURCE:                   PCT Int. Appl., 799 pp.
                          CODEN: PIXXD2
DOCUMENT TYPE:            Patent
LANGUAGE:                 English
FAMILY ACC. NUM. COUNT:   5
PATENT INFORMATION:

     PATENT NO.           KIND   DATE        APPLICATION NO.          DATE
     ---------------      ----   --------    --------------------     --------
     WO 2004037996         A2    20040506    WO 2003-US33656          20031024
     WO 2004037996         A3    20041229
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD,
             GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
             LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO,
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NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG

US 2004083084 A1 20040429 US 2002-291878 20021112
WO 2004044839 A2 20040527 WO 2002-US38216 20021112
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH,
CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD,
GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ,
LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL,
TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ,
BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
US 2004106113 A1 20040603 US 2002-291886 20021112
PRIORITY APPLN. INFO.: US 2002-420729P P 20021024
US 2002-421062P P 20021025
US 2002-421102P P 20021025
US 2002-424701P P 20021108
US 2002-424715P P 20021108
US 2002-424718P P 20021108
US 2002-291878 A 20021112
US 2002-291886 A 20021112
US 2002-425256P P 20021112
WO 2002-US38216 A 20021112
WO 2002-US38222 A 20021112
US 2003-448461P P 20030221
US 2003-448462P P 20030221
US 2003-457877P P 20030327
US 2003-458373P P 20030331

AB The present invention relates generally to a method for evaluating and/or predicting breast cancer states and outcomes by measuring gene and metagene expression levels and integrating such data with clin, risk factors. Genes and metagenes whose expressions are correlated with a particular breast cancer risk factor or phenotype are provided using binary prediction tree modeling. The invention provides 175 genes associated with metagene predictors of lymph node metastasis, 216 genes associated with metagene predictors of breast cancer recurrence, and 496 metagenes related to breast cancer study. Methods of using

the subject genes and metagenes in diagnosis and treatment methods, as well as drug screening methods, etc are also provided. In addition, reagents, media and kits that find use in practicing the subject methods are also provided.

L9 ANSWER 2 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 28 Nov 2003
ACCESSION NUMBER: 2003:927817 CAPLUS
DOCUMENT NUMBER: 140:107208
TITLE: α- **Lactalbumin** unfolding is not sufficient to cause apoptosis, but is required for the conversion to HAMLET (human α- **lactalbumin** made lethal to tumor cells)
AUTHOR(S): Svensson, Malin; Fast, Jonas; Mossberg, Ann-kristin; Dueringer, Caroline; Gustafsson, Lotta; Hallgren, Oskar; Brooks, Charles L.; Berliner, Lawrence; Linse, Sara; Svanborg, Catharina
CORPORATE SOURCE: Department of Microbiology, Immunology and Glycobiology (MIG), Institute of Laboratory Medicine, Lund University, Lund, Swed.
SOURCE: Protein Science (2003), 12(12), 2794-2804
CODEN: PRCIEI; ISSN: 0961-8368
PUBLISHER: Cold Spring Harbor Laboratory Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB HAMLET (human α- **lactalbumin** made lethal to tumor cells) is a complex of human α- **lactalbumin** (I) and oleic acid (C18:1:9 cis) that kills tumor cells by an apoptosis-like mechanism. Previous studies have shown that a conformational change is required to form HAMLET from I, and that a partially unfolded conformation is maintained in the HAMLET complex. This study examined if unfolding of I was sufficient to induce cell death. The authors used bovine I Ca2+-binding site **mutant D87A**, which is unable to bind Ca2+, and thus remains partially unfolded regardless of solvent conditions. The **D87A mutant** protein was found to be inactive in the apoptosis assay, but could readily be converted to a HAMLET-like complex in the presence of oleic acid. BAMLET (bovine α- **lactalbumin** made lethal to tumor cells) and **D87A**-BAMLET complexes were both able to kill tumor cells. This activity was independent of the Ca2+ site, as HAMLET maintained a high affinity for Ca2+ but **D87A**-BAMLET was active with no Ca2+ bound. It was concluded that partial unfolding of I is necessary but not sufficient to trigger cell death, and that the activity of HAMLET is defined both by the protein and the lipid cofactor. Furthermore, a functional Ca2+-binding site is not required for conversion of I to the active complex or to cause cell death. This suggests that the lipid cofactor stabilizes the altered fold without interfering with the Ca2+ site.

IT **7440-70-2, Calcium,** biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(a functional Ca2+-binding site in α- **lactalbumin** is not required for its conversion to HAMLET (human α- **lactalbumin** made lethal to tumor cells) or to induce apoptosis)

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 3 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 21 Nov 2003
ACCESSION NUMBER: 2003:913191 CAPLUS
DOCUMENT NUMBER: 139:375001
TITLE: Active complex of α- **lactalbumin** (HAMLET) and cofactor for the treatment of papillomas
INVENTOR(S): Svanborg, Catherine
PATENT ASSIGNEE(S): Swed.
SOURCE: PCT Int. Appl., 22 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003095490	A1	20031120	WO 2003-IB2366	20030508
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1506233	A1	20050216	EP 2003-727868	20030508
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				

PRIORITY APPLN. INFO.: GB 2002-10464 A 20020508
WO 2003-IB2366 W 20030508

AB The invention discloses the use of a biol. active complex of α-**lactalbumin**, selected from HAMLET (human α-**lactalbumin** made lethal to tumor cells) or a biol. active modification thereof, or a biol. active fragment of either of these, in the preparation of a medicament for use in the treatment of papillomas, e.g. cutaneous papillomas.

IT **7440-70-2**, **Calcium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(and **calcium** binding site; active complex of α-**lactalbumin** (HAMLET) and cofactor for papilloma treatment)

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 14 Sep 2003
ACCESSION NUMBER: 2003:719503 CAPLUS
DOCUMENT NUMBER: 139:224401
TITLE: Biologically active complex
INVENTOR(S): Svanborg, Catharina; Svensson, Malin Wilhelmina
PATENT ASSIGNEE(S): Swed.
SOURCE: PCT Int. Appl., 56 pp.

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003074547	A2	20030912	WO 2003-IB1293	20030307
WO 2003074547	A3	20031127		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
EP 1485413	A2	20041215	EP 2003-710101	20030307
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				

PRIORITY APPLN. INFO.: GB 2002-5347 A 20020307
WO 2003-IB1293 W 20030307

AB A biol. active complex comprising alpha-**lactalbumin** or a variant of alpha-**lactalbumin** which is in the apo folding state, or a fragment of either of any of these, and a cofactor which stabilizes the complex in a biol. active form, provided that any fragment of alpha-**lactalbumin** or a variant thereof comprises a region corresponding to the region of α- **lactalbumin** which forms the interface between the alpha and beta domains, and further provided that when the complex comprises native alpha-**lactalbumin**, the cofactor is other than C18:1:9 cis fatty acid. These complexes have therapeutic applications for example in the treatment of cancer and as antibacterial agents.

IT **7440-70-2, Calcium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(biol. active complex of α- **lactalbumin** and cofactor such as cis-fatty acids as anticancer and antibacterial agents in relation to removal of **calcium** ions or **calcium** binding site)

L9 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 28 Jul 2003
ACCESSION NUMBER: 2003:574056 CAPLUS
DOCUMENT NUMBER: 139:333677
TITLE: A MvaI PCR-RFLP detecting a silent allele at the goat α- **lactalbumin** locus
AUTHOR(S): Cosenza, Gianfranco; Gallo, Daniela; Illario, Rosa; Di Gregorio, Paola; Senese, Carmela; Ferrara, Lino; Ramunno, Luigi
CORPORATE SOURCE: Dipartimento di Scienze Zootecniche ed Ispezione degli Alimenti, Universita degli Studi di Napoli "Federico II", Portici, Italy
SOURCE: Journal of Dairy Research (2003), 70(3), 355-357

CODEN: JDRSAN; ISSN: 0022-0299
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Alpha-**lactalbumin** (α-la), a **calcium** metalloprotein, is one of the major serum-proteins in ruminant milk (Jenness, 1982) and induces lactose synthesis in the mammary gland by interacting with the enzyme UDP-galactosyltransferase, giving rise to the heterodimer enzyme lactose synthase (Ebner & Brodbeck, 1968; Kuhn, 1983). The goat α-la transcription unit (LALBA), located on chromosome 5 (Hayes et al. 1993), is organized in 4 exons varying in length from 75 nucleotides (3rd exon) to 329 nucleotides (4th exon) coding for a 123-amino acid polypeptide chain (Vilotte et al. 1991). According to the strong similarity between bovine α-la (Vilotte et al. 1987) and human lysozyme (similar mol. weight, the same number of S-S bonds, identical N and C terminal residues; Peters et al. 1989), it has been proposed that both genes arose from a common ancestor (Vilotte et al. 1991). The authors reported the identification of a silent allele at the goat LALBA locus and describes a method based on PCR-RFLP for its detection.
REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 20 Nov 2002
ACCESSION NUMBER: 2002:878224 CAPLUS
DOCUMENT NUMBER: 138:217230
TITLE: Structural and thermodynamic architecture of the Ca2+ binding site engineered into human lysozyme
AUTHOR(S): Kuroki, Ryota; Shigematsu, Hideki
CORPORATE SOURCE: Pharmaceutical Research Laboratories, Kirin Brewery Co., Ltd., Kanazawa-ku, Yokohama, 236-0004, Japan
SOURCE: Recent Research Developments in Protein Engineering (2001), 1(Pt. 2), 197-212
CODEN: RRDPCU
PUBLISHER: Research Signpost
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with refs. Structural determinants of Ca2+ binding sites within proteins typically comprise several acidic residues in appropriate juxtaposition. The α- **lactalbumins** and some natural lysozymes are known to have a Ca2+ binding site consisting of at least three aspartic acids and two main chain carbonyls located between two helixes. Three residues (Ala-83, Gln-86 and Ala-92) in human lysozyme are characteristically replaced by **Lys**, Asp, and Asp, resp., in natural Ca2+ binding lysozymes and α-**lactalbumins**. To investigate the architecture of the Ca2+ binding site, **mutagenesis** has been introduced into human lysozyme, and the subsequent effect of these **mutations** on the stability and Ca2+ binding properties of human lysozyme was analyzed by X-ray crystallog. and calorimetry. Although neither point **mutant** (Glu-86→Asp or Ala-92→Asp) showed Ca2+ affinity, the **mutant** with both these **mutations** clearly showed Ca2+ binding affinity, indicating that both residues are essential. The further **mutation** of Ala-83→**Lys** did not affect the Ca2+ binding of the double **mutant**. The Ca2+ binding site engineered into human lysozyme showed a pentagonal

bipyramidal structure consisting of three aspartic acids at positions 86, 91, and 92, which was similar to that of α-**lactalbumin**. The point **mutations** Ala-83→Lys and Glu-86→Asp did not affect the stability of lysozyme, whereas the **mutation** Ala-92→Asp rendered lysozyme about 1.3 kcal/mol less stable. Structural analyses showed that both Asp-86 and Lys-83 were exposed to solvent in the absence of Ca2+. Side chains of Asp-86 and Asp-91 were rotated in opposite directions about the χ1 angle, as if to reduce the electrostatic repulsion. Three charged amino acids introduced into the Ca2+ binding site did not significantly affect stability of the human lysozyme. Local conformational change of the side chains may be enough to remove the effect of charge repulsion.

IT **7440-70-2, Calcium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(structural and thermodn. architecture of Ca2+ binding site engineered into human lysozyme)

REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 7 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 11 Aug 2002
ACCESSION NUMBER: 2002:596514 CAPLUS
DOCUMENT NUMBER: 137:306587
TITLE: Studies on the metal binding sites in the catalytic domain of β1,4-galactosyltransferase
AUTHOR(S): Boeggeman, Elizabeth; Qasba, Pradman K.
CORPORATE SOURCE: Structural Glycobiology Section and Intramural Research Support Program-SAIC, Laboratory of Experimental and Computational Biology, NCI-CCR, Frederick, MD, 21702-1201, USA
SOURCE: Glycobiology (2002), 12(7), 395-407
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The catalytic domain of bovine β1,4-galactosyltransferase (β4Gal-T1) has been shown to have two metal binding sites, each with a distinct binding affinity. Site I binds Mn2+ with high affinity and does not bind Ca2+, whereas site II binds a variety of metal ions, including Ca2+. The catalytic region of β4Gal-T1 has DXD motifs, associated with metal binding in glycosyltransferases, in two sep. sequences: D242YDYNCFVFSDVD254 (region I) and W312GWGGEDDD320 (region II). Recently, the crystal structure of β4Gal-T1 bound with UDP, Mn2+, and α- **lactalbumin** was determined in our laboratory It shows that in the primary metal binding site of β4Gal-T1, the Mn2+ ion, is coordinated to five ligands, two supplied by the phosphates of the sugar nucleotide and the other three by Asp254, His347, and Met344. The residue Asp254 in the D252VD254 sequence in region I is the only residue that is coordinated to the Mn2+ ion. Region II forms a loop structure and contains the E317DDD320 sequence in which residues Asp318 and Asp319 are directly involved in GlcNAc binding. This study, using site-directed **mutagenesis**, kinetic, and binding affinity anal., shows that Asp254 and His347 are strong metal ligands, whereas Met344, which coordinates less strongly, can be substituted by alanine or glutamine. Specifically, substitution of Met344 to Gln has a less severe effect

on the catalysis driven by Co2+. Glu317 and Asp320 **mutants**, when partially activated by Mn2+ binding to the primary site, can be further activated by Co2+ or inhibited by Ca2+, an effect that is the opposite of what is observed with the wild-type enzyme.

IT **7440-70-2, Calcium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study) (metal binding sites in catalytic domain of β1,4-galactosyltransferase)

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 28 May 2002
ACCESSION NUMBER: 2002:394395 CAPLUS
DOCUMENT NUMBER: 137:151470
TITLE: A metal binding in the polypeptide chain improves the folding efficiency of a denatured and reduced protein
AUTHOR(S): Ohkuri, Takatoshi; Ueda, Tadashi; Yoshida, Yuichiro; Abe, Yoshito; Hamasaki, Naotaka; Imoto, Taiji
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan
SOURCE: Biopolymers (2002), 64(2), 106-114
CODEN: BIPMAA; ISSN: 0006-3525
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In order to examine the effect of a metal binding to the polypeptide chain on the aggregation of a protein in the refolding process, we prepared a **mutant** hen lysozyme possessing the same Ca2+ binding site as in human α- **lactalbumin** by Escherichia coli expression system (Ser-1 CaB lysozyme). In the presence of 2 mM CaCl2, the refolding yield of Ser-1 CaB lysozyme at a low protein concentration (25 μg/mL) was similar to that of the wild-type lysozyme (80%), but that at high protein concentration (200 μg/mL) decreased (15%) due to aggregation comparing to that of the wild-type lysozyme (45%). However, the refolding yield of Ser-1 CaB lysozyme in the presence of 100 mM CaCl2 even at a protein concentration of 200 μg/mL was 80% and was higher than that of the wild-type lysozyme. From anal. of chemical shift changes of the cross peaks in the backbone region of total correlated spectroscopy (TOCSY) spectra of a decapeptide possessing the same **calcium** binding site as in Ser-1 CaB lysozyme in the presence of various concns. of Ca2+, it was suggested that the dissociation constant of Ca2+-peptide complex was estimated to be 20-36 mM. Moreover, the solubility of the denatured Ser-1 CaB lysozyme in the presence of 100 mM CaCl2 was higher than that in the presence of 2 mM CaCl2 whereas the solubility of the denatured Ser-1 lysozyme in the presence of 100 mM CaCl2 was not higher than that in the presence of 2 mM CaCl2. Therefore, it was concluded that the reduced lysozyme possessing the Ca2+ binding site was efficiently folded in the presence of high concentration of Ca2+ (100 mM) even at high protein concentration due to depression of aggregation by the binding of Ca2+ to the polypeptide chain in Ser-1 CaB lysozyme.

IT **7440-70-2, Calcium**, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study) (improvement of folding efficiency of modified lysozyme mols. by

calcium binding)
REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 9 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 30 Dec 2001
ACCESSION NUMBER: 2001:936216 CAPLUS
DOCUMENT NUMBER: 136:146798
TITLE: **Mutating** aspartate in the **calcium**-binding site of α-**lactalbumin**: effects on the protein stability and cation binding
AUTHOR(S): Permyakov, Sergei E.; Uversky, Vladimir N.; Veprintsev, Dmitry B.; Cherskaya, Alexandra M.; Brooks, Charles L.; Permyakov, Eugene A.; Berliner, Lawrence J.
CORPORATE SOURCE: Institute for Biological Instrumentation of the Russian Academy of Sciences, Pushchino, 142290, Russia
SOURCE: Protein Engineering (2001), 14(10), 785-789
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The residue Asp87, which is in the **calcium**-binding loop of bovine α- **lactalbumin** (α-LA) and provides a side-chain carboxylate oxygen for ligand **Ca**(II) co-ordination, was substituted by either alanine or asparagine. The phys. properties and **calcium**-binding affinities were monitored by intrinsic fluorescence and CD spectroscopy. **D87A** α-LA displayed a total loss of rigid tertiary structure, a dramatic loss in secondary structure and negligible **calcium** affinity. On the contrary, **D87N** α-LA displayed native-like secondary structure with a somewhat destabilized tertiary structure. When the well-documented N-terminal methionine was enzymically removed from **D87N** α-LA, the structure appeared to more closely resemble native α-LA. Remarkably, the thermal transition mid-temperature of apo-desMetD87N α-LA was .apprx.31° vs. native apo- α-LA (.apprx.25°), probably due to neg. charge "compensation" in the **calcium** co-ordination site. On the other hand, the transition mid-temperature of Ca(II)-bound desMetD87N α-LA was .apprx.57° vs. native α-LA (.apprx.66°), which was related to a decreased Ca(II) affinity (K = .apprx.2.1+105 vs. .apprx.1.7+107/M at 40°, resp.). These results reaffirm that alanine substitution in site specific **mutagenesis** is not always a prudent choice. Substitutions must be conservative with only minimal changes in functional groups and side-chain volume
IT **7440-70-2, Calcium,** biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(**mutating** aspartate in the **calcium**-binding site of α- **lactalbumin**: effects on the protein stability and cation binding)
REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN

ED Entered STN: 23 Jul 2001
ACCESSION NUMBER: 2001:527562 CAPLUS
DOCUMENT NUMBER: 135:343570
TITLE: Chemical composition and percent distribution of caseins and whey proteins of milk of Italian Friesian cows with different genotype for α-**lactalbumin** (+15) **polymorphism**
AUTHOR(S): Dall'Olio, S.; Davoli, R.; Mariani, P.; Summer, A.; Tirelli, A.; Milc, J.; Russo, V.
CORPORATE SOURCE: Dipt. Prot. Valor. Agroalimentare, Sezione di Allevamenti Zootecnici, Reggio Emilia, 42100, Italy
SOURCE: Scienza e Tecnica Lattiero-Casearia (2001), 52(2), 115-126
CODEN: SLCAAF; ISSN: 0390-6361
PUBLISHER: Associazione Italiana Tecnici del Latte
DOCUMENT TYPE: Journal
LANGUAGE: Italian
AB The protein composition characteristics of 20 individual milk samples from 10 pairs of Italian Friesian cows were studied in 6 dairy herds in the province of Reggio Emilia. Each pair was formed by a cow with α-La (+15) A/A genotype and a cow with α-La (+15) B/B genotype, both at a similar stage of lactation and parity. The milk samples were analyzed for electrophoretic % distribution of caseins and 4 whey proteins (α- **lactalbumin**, β-lactoglobulin, serum albumin, Ig) by reversed-phase HPLC. The main physicochem. characteristics, total N content, minerals, and milk coagulation parameters were also determined The two α-La (+15) homozygous genotype groups had similar casein and β-lactoglobulin genotype distribution. Each milk sample had normal values for the indicator parameters of the udder health (somatic cells, lactose, chloride). The A/A cows compared to B/B cows had higher average levels of N proteose-peptone (12.4±4.1 vs. 8.1 ± 3.2 mg N/100 g), probably due to higher β-casein levels.
IT **7440-70-2, Calcium,** biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); FFD (Food or feed use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(milk casein and proteins % distribution in Italian Friesian dairy cows with different genotype for α- **lactalbumin polymorphism**)
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 11 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 10 Jan 2001
ACCESSION NUMBER: 2001:19917 CAPLUS
DOCUMENT NUMBER: 134:173487
TITLE: Use of protein engineering methods for studies of **Calcium**-binding proteins
AUTHOR(S): Permyakov, S. E.; Permyakev, E. A.
CORPORATE SOURCE: Institute of Biological Instrumentation, Russian Academy of Sciences, Pushchino, 142290, Russia
SOURCE: Biofizika (2000), 45(6), 990-1006
CODEN: BIOFAI; ISSN: 0006-3029
PUBLISHER: Nauka
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Russian

AB A review with 85 refs. Major results of the use of protein engineering methods in studies of **calcium**-binding proteins with the highest affinity for **calcium** and known three-dimensional structure (parvalbumin, calmodulin, troponin C, calbindin, recoverin, α- **lactalbumin**, and others) are presented. Specific features of recombinant **calcium**-binding proteins are discussed. Expts. with genetic introduction of fluorescent probes, tryptophan and tyrosine, into proteins are overviewed. Effects of **mutations** in different parts of protein mols. (**calcium**-binding loops, hydrophobic core, and others) on their structure and properties and attempts of creation of artificial **calcium**-binding sites are discussed.

L9 ANSWER 12 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 19 Apr 2000
ACCESSION NUMBER: 2000:248972 CAPLUS
DOCUMENT NUMBER: 133:13880
TITLE: Peptide analogs from E-cadherin with different **calcium**-binding affinities
AUTHOR(S): Yang, W.; Tsai, T.; Kats, M.; Yang, J. J.
CORPORATE SOURCE: Department of Biology, Georgia State University, Atlanta, GA, 30303, USA
SOURCE: Journal of Peptide Research (2000), 55(3), 203-215
CODEN: JPERFA; ISSN: 1397-002X
PUBLISHER: Munksgaard International Publishers Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Cadherins are a family of **calcium**-dependent cell-surface proteins that are fundamental in controlling the development and maintenance of tissues. Motif B of E-cadherin seems to be a crucial **calcium**-binding site as single point **mutations** (D134A and D134K) completely inactivate its adhesion activity. We analyzed peptide models corresponding to motif B (amino acids 128-144) as well as selected **mutations** of this motif. Our NMR studies showed that this motif B sequence is actually an active **calcium**-binding region, even in the absence of the rest of the cadherin mol. We found that the binding affinity of this motif is very sensitive to **mutations**. For example, our peptide P128-144 with the native **calcium**-binding sequence has an affinity of Kd 0.4 mM, whereas the **mutants** P128-144/D134A and P128-144/D134K containing the replacement of Asp134 by Ala and Lys, have Kd values of only 1.5 and 11 mM, resp. Removing Asp at position 134, which correlates with the loss of adhesion activity, decreases **calcium**-binding affinity 20-fold. Ala132, along with residues Asp134, Asp136 and Asn143, is involved in **calcium** binding in solution We also demonstrated that the **calcium**-binding affinity can be increased ≈ 3-fold when an addnl. Asp is introduced at position 132. In 50% organic solvent, this binding affinity of peptide P128-144/A132D (17-mer) from E-cadherin is similar to that of peptide P72-100/C73-77-91A (29-mer) from α- **lactalbumin**.

IT **7440-70-2, Calcium**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(peptide analogs from E-cadherin with different **calcium**-binding affinities)

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 13 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 09 Sep 1999
ACCESSION NUMBER: 1999:569919 CAPLUS
DOCUMENT NUMBER: 131:296742
TITLE: Fine tuning the N-terminus of a **calcium** binding protein: α- **lactalbumin**
AUTHOR(S): Veprintsev, Dmitry B.; Narayan, Mahesh; Permyakov, Serge E.; Uversky, Vladimir N.; Brooks, Charles L.; Cherskaya, Alexandra M.; Permyakov, Eugene A.; Berliner, Lawrence J.
CORPORATE SOURCE: Institute for Biological Instrumentation, Russian Academy of Science, Pushchino, Russia
SOURCE: Proteins: Structure, Function, and Genetics (1999), 37(1), 65-72
CODEN: PSFGEY; ISSN: 0887-3585
PUBLISHER: Wiley-Liss, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The effects of amino acid substitutions in the N-terminus of bovine recombinant α- **lactalbumin** (including enzymic removal of the N-terminal methionine and deletion of Glu-1) were studied by intrinsic fluorescence, CD, and differential scanning microcalorimetry (DSC). Wild-type recombinant α- **lactalbumin** has a lower thermostability and **calcium** affinity compared to the native protein, while the properties of wild-type protein with the N-terminal methionine enzymically removed are similar to the native protein. Taken together, the fluorescence, CD, and DSC results show that recombinant wild type α- **lactalbumin** in the absence of **calcium** ion is in a type of molten globule state. The delta-E1 **mutant**, where the Glu1 residue of the native sequence is genetically removed, leaving an N-terminal methionine in its place, shows almost one order of magnitude higher affinity for **calcium** and higher thermostability (both in the absence and presence of **calcium**) than the native protein isolated from milk. It was concluded that the N-terminus of the protein dramatically affects both stability and function as manifested in **calcium** affinity.
IT **7440-70-2, Calcium**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(N-terminus of **calcium** binding protein α- **lactalbumin** dramatically affects thermostability and **calcium** affinity)
REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 14 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 06 Jan 1999
ACCESSION NUMBER: 1999:7316 CAPLUS
DOCUMENT NUMBER: 130:164819
TITLE: Structural and thermodynamic responses of **mutations** at a Ca2+ binding site engineered into human lysozyme
AUTHOR(S): Kuroki, Ryota; Yutani, Katsuhide
CORPORATE SOURCE: Central Laboratories for Key Technology, **Kirin** Brewery Co. Ltd., Yokohama, 236, Japan
SOURCE: Journal of Biological Chemistry (1998), 273(51),

34310-34315
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Structural determinants of Ca2+ binding sites within proteins typically comprise several acidic residues in appropriate juxtaposition. Three residues (Ala-83, Gln-86, and Ala-92) in human lysozyme are characteristically **mutated** to Lys, Asp, and Asp, resp., in natural Ca2+ binding lysozymes and α-**lactalbumins**. The effects of these **mutations** on the stability and Ca2+ binding properties of human lysozyme were investigated using calorimetry and were interpreted with crystal structures. The double **mutant**, in which Glu-86 and Ala-92 were replaced with Asp, clearly showed Ca2+ binding affinity, whereas neither point **mutant** showed Ca2+ affinity, indicating that both residues are essential. The further **mutation** of Ala-83 → Lys did not affect the Ca2+ binding of the double **mutant**. The point **mutations** Ala-83 → Lys and Glu-86 → Asp did not affect the stability, whereas the **mutation** Ala-92 → Asp was about 1.3 kcal/mol less stable. Structural analyses showed that both Asp-86 and Lys-83 were exposed to solvent. Side chains of Asp-86 and Asp-91 were rotated in opposite directions about χ1 angle, as if to reduce the electrostatic repulsion. The charged amino acids at the Ca2+ binding site did not significantly affect stability of the protein, possibly because of the local conformational change of the side chains.

IT **7440-70-2, Calcium**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(site-directed **mutagenesis** in **calcium** site of human lysozyme and the effects on stability and **calcium** affinity in the **mutant** enzymes)

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 13 Sep 1997
ACCESSION NUMBER: 1997:584594 CAPLUS
DOCUMENT NUMBER: 127:187204
TITLE: Functional identification of **calcium** binding residues in bovine α-**lactalbumin**
AUTHOR(S): Anderson, Patricia J.; Brooks, Charles L.; Berliner, Lawrence J.
CORPORATE SOURCE: Departments of Chemistry and Veterinary Biosciences, Ohio State University, Columbus, OH, 43210, USA
SOURCE: Biochemistry (1997), 36(39), 11648-11654
CODEN: BICHAW; ISSN: 0006-2960
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The functional role of previously identified Ca2+-binding residues in α- **lactalbumin** (α-LA) was investigated by site-directed **mutagenesis**. **Mutation** of Asp-82 to Ala did not affect the binding affinity for Ca2+, the protein

structure, or its function in the lactose synthase assay, suggesting that this Asp side-chain was not essential for Ca2+ binding or structural stabilization. In contrast, **mutation** of either Asp-87 or Asp-88 to Ala completely eliminated the strong Ca2+ binding and altered α-LA as shown by several spectroscopically derived properties such as near- and far-UV CD and intrinsic fluorescence studies. These latter 2 **mutants** displayed significantly reduced abilities to stimulate lactose synthase activity (<3.5% of the maximal rate). Addnl., residues Lys-79 and Asp-84, which chelate Ca2+ by backbone carbonyls, were **mutated** to Ala. **Mutant** K79A lost .apprx.50% of its tertiary structure and stability (as determined by CD) but retained full Ca2+ binding activity, indicating that at least the Lys side-chain does not influence the carbonyl-mediated Ca2+ coordination. In contrast, **mutant** D84A lost .apprx.25% of its tertiary structure and stability which was accompanied by a modest reduction in Ca2+ affinity. Both **mutants** were able to stimulate normal lactose synthase activity. The triple **mutant**, D82A/ **D87A**/D88A α-LA, lost its ability to bind Ca2+, similar to **D87A** and D88A. These studies clearly demonstrate the importance and variation of side-chain interactions, which might be the seminal event in the establishment of the correct Ca2+-binding loop conformation, possibly to stabilization and final folding of the overall protein structure.

IT **7440-70-2, Calcium**, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(functional identification of Ca2+-binding residues in bovine α- **lactalbumin**)

L9 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 26 Jul 1997
ACCESSION NUMBER: 1997:470875 CAPLUS
DOCUMENT NUMBER: 127:94226
TITLE: Protein composition of goat's milk: its particularities
AUTHOR(S): Martin, P.
CORPORATE SOURCE: Lab. Genetique Biochimique Cytogenetique, INRA, Jouy en Josas, 78352, Fr.
SOURCE: Colloques - Institut National de la Recherche Agronomique (1997), 81(Interets Nutritionnel et Dietetique du Lait de Chevre), 27-49
CODEN: COLIEZ; ISSN: 0293-1915
PUBLISHER: Institut National de la Recherche Agronomique
DOCUMENT TYPE: Journal; General Review
LANGUAGE: French

AB A review with 37 refs. The protein fraction of milk differs according to the species qual. and quant. It comprises more than thirty different mol. species arising from the expression of six structural genes encoding six different peptide chains, including two main whey proteins (α- **lactalbumin** and β-lactoglobulin) and the four caseins (αs1, αs2, β, and κ) which interact, in the presence of **calcium** phosphate, to form micelles. As for cow milk, which remains the reference, the protein fraction of goat milk displays a high casein content (around 80%). Post translational modifications (phosphorylation, glycosylation, proteolysis) affecting primarily caseins are, in part, responsible for the multiplicity of protein forms observed A pronounced and sometimes unusual genetic **polymorphism**, including quant. variability further add to this complexity. This is particularly true for

αs1-casein of which seven protein variants associated with four levels of synthesis ranging between 0 and 3.6 g/L (per allele) have been found in the goat species. Variants associated with a low level of synthesis are internally deleted due to anomalous splicing. Thus, it is theor. possible to modulate, by sorting goats according to their genotypes, the composition of goat milk and to adapt their physico-chemical properties to the desired values. Beside α- **lactalbumin** and β-lactoglobulin, the whey protein fraction comprises a large number of protein components, at low concns. but ensuring crucial functions, including local protection of the mammary mucosa in the mother and/or buccal and intestinal mucosa in the newborn. These proteins include Igs (IgA, IgG), lactoferrin (in larger amts. in human milk), and enzymes with bactericidal/bacteriostatic activities, such as lactoperoxidase and lysozyme. Caseins also yield biol. active peptides able to mediate some metabolic, neuroendocrine, and immunol. functions. The structural variability within (induced by genetic **polymorphism** and anomalous splicing) or between species may provide a large diversity of original peptides of which putative roles remain to be identified.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 11 Apr 1997
ACCESSION NUMBER: 1997:235299 CAPLUS
DOCUMENT NUMBER: 126:327368
TITLE: Structure/function studies of **mutants** of alpha-**lactalbumin** and alpha-thrombin (molten globule, **calcium** binding, hirudin)
AUTHOR(S): Anderson, Patricia Jane
CORPORATE SOURCE: Ohio State Univ., Columbus, OH, USA
SOURCE: (1996) 210 pp. Avail.: Univ. Microfilms Int., Order No. DA9710520
From: Diss. Abstr. Int., B 1997, 57(10), 6230
DOCUMENT TYPE: Dissertation
LANGUAGE: English
AB Unavailable
IT **7440-70-2, Calcium,** biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(binding; structure/function studies of **mutants** of α- **lactalbumin** and α-thrombin)

L9 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 07 Dec 1996
ACCESSION NUMBER: 1996:718405 CAPLUS
DOCUMENT NUMBER: 126:3316
TITLE: Thermodynamic Characterization of the Partially Unfolded State of Ca2+-Loaded Bovine α-**Lactalbumin**: Evidence That Partial Unfolding Can Precede Ca2+ Release
AUTHOR(S): Vanderheeren, Geertrui; Hanssens, Ignace; Meijberg, Wim; Van Aerschot, Arthur
CORPORATE SOURCE: Interdisciplinary Research Center, Katholieke Universiteit Leuven, Kortrijk, B-8500, Belg.
SOURCE: Biochemistry (1996), 35(51), 16753-16759
CODEN: BICHAW; ISSN: 0006-2960

PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The thermal denaturation of bovine α- **lactalbumin** (BLA) was studied at pH 7.5 and at various Ca2+ concns. using near-UV CD and differential scanning calorimetry. The Ca2+ dependence of the denaturation equilibrium proves that, in the transition region, partially unfolded α- **lactalbumin** consists of a mixture of Ca2+-loaded and Ca2+-free protein. The thermodn. parameters of the unfolding of these two species were determined at 68 °C and were then compared with one another, with the thermodn. parameters deduced from calorimetric titration of α- **lactalbumin** with Ca2+, and with those derived from Ca2+ titration of a **mutant** human lysozyme having an engineered Ca2+-binding site. This comparison indicated that (a) the unfolding curves for Ca2+-BLA deduced from the near-UV ellipticity change are more able to distinguish between unfolding with and without Ca2+ release than those deduced from differential scanning calorimetry, (b) the Ca2+-loaded denatured state of BLA is more folded than the Ca2+-free protein at 68 °C, and (c) a heat-induced unfolding process, consisting of an initial Ca2+ release, followed by a conformational relaxation, is unlikely to occur at the exptl. pH and in the millimolar region of Ca2+ concns., due to the large free energy requirement of the initial step. A more probable mechanism would be unfolding via a Ca2+-loaded intermediately unfolded state, with subsequent Ca2+ release.
IT **7440-70-2, Calcium,** biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(evidence from thermodn. characterization of partially unfolded state of Ca2+-loaded bovine α- **lactalbumin** that partial unfolding can precede Ca2+ release)
REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 20 Aug 1994
ACCESSION NUMBER: 1994:474785 CAPLUS
DOCUMENT NUMBER: 121:74785
TITLE: Creation and phenotypic analysis of α-**lactalbumin**-deficient mice
AUTHOR(S): Stinnakre, M. G.; Vilotte, J. L.; Mercier, J. C.
CORPORATE SOURCE: Laboratoire de Genetique Biochimique et de Cytogenetique, Institut National de la Recherche Agronomique, Jouy-en-Josas, 78352, Fr.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1994), 91(14), 6544-8
CODEN: PNASA6; ISSN: 0027-8424
DOCUMENT TYPE: Journal
LANGUAGE: English
AB α- **Lactalbumin** is an abundant milk-specific **calcium** metalloprotein which has an evolutionary relationship to lysozyme. It modifies the substrate specificity of a Golgi galactosyltransferase by forming the lactose synthetase binary complex. Lactose, together with other sugar and diffusible ions, is responsible for the osmotic pressure of milk. To assess the involvement of α- **lactalbumin** in lactogenesis, α-**lactalbumin**-deficient mice were created by disrupting the gene

by homologous recombination in embryonic stem cells. Homozygous **mutant** mice are viable and fertile but females cannot feed their offspring. They produce a highly viscous milk that pups appear to be unable to remove from the mammary gland. This milk is rich in fat and protein and is devoid of α- **lactalbumin** and lactose. The phenotype of heterozygous mice was found to be intermediate, with a 40% decrease in α- **lactalbumin** but only a 10-20% decrease in the lactose content of their milk compared with wild-type animals. These results emphasize the key function of α- **lactalbumin** in lactogenesis and open new opportunities to manipulate milk composition

L9 ANSWER 20 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 22 Jan 1994
ACCESSION NUMBER: 1994:26290 CAPLUS
DOCUMENT NUMBER: 120:26290
TITLE: Stability effects associated with the introduction of a partial and a complete **calcium** -binding site into human lysozyme
AUTHOR(S): Haezebrouck, Petra; De Baetselier, Annie; Joniau, Marcel; Van Dael, Herman; Rosenberg, Steve; Hanssens, Ignace
CORPORATE SOURCE: Interdiscip. Res. Cent., Kathol. Univ. Leuven, Kortrijk, B-8500, Belg.
SOURCE: Protein Engineering (1993), 6(6), 643-9
CODEN: PRENE9; ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two **mutants** of human lysozyme were synthesized. **Mutant** A92D, in which Ala92 was substituted by Asp, contains a partial Ca2+-binding site and **mutant** M4, in which Ala83, Gln86, Asn88 and Ala92 were replaced by Lys, Asp, Asp and Asp, resp., contains the complete Ca2+-binding site of bovine α- **lactalbumin**. The Ca2+-binding consts. of wild type human lysozyme and of **mutants** A92D and M4, measured at 25°C and pH 7.5, were 2(±1) + 102 M-1, 8(±2) + 103 M-1 and 9(±0.5) + 106 M-1, resp. Information gathered from microcalorimetric and CD spectroscopic measurements indicates that the conformational changes in the M4 **mutant** lysozyme, induced by Ca2+ binding, are smaller than those observed for bovine α- **lactalbumin** and for the Ca2+-binding equine lysozyme. At pH 4.5, the thermostability of both the apo and Ca2+ forms of the A92D human was decreased in comparison with that of native human lysozyme. In particular, within the apo form of this **mutant** an α-helix-containing sequence was destabilized. In contrast, at the same pH the thermostability of the apo and Ca2+ forms of the M4 **mutant** lysozyme was increased. The ε-ammonium group of the Lys83 side chain is assumed to be responsible for the stabilization of the apo form of this **mutant**.
IT **7440-70-2, Calcium**, biological studies
RL: BIOL (Biological study)
(lysozyme wild-type and partial and complete **calcium** -binding site **mutants** of human affinity for and conformation response to)

L9 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 16 Feb 1993
ACCESSION NUMBER: 1993:54782 CAPLUS
DOCUMENT NUMBER: 118:54782

TITLE: Hydrophobic interaction of lysozyme and α-**lactalbumin** from equine milk whey
AUTHOR(S): Haezebrouck, Petra; Noppe, Wim; Van Dael, Herman; Hanssens, Ignace
CORPORATE SOURCE: Interdiscip. Res. Cent., KUL, Kortrijk, Belg.
SOURCE: Biochimica et Biophysica Acta (1992), 1122(3), 305-10
CODEN: BBACAQ; ISSN: 0006-3002
DOCUMENT TYPE: Journal
LANGUAGE: English

AB From fluorescence measurements on mixts. of 5,5'-bis(1,8-anilinonaphthalenesulfonic acid) (bis-ANS) and equine lysozyme and from Ca2+-dependent hydrophobic interaction chromatog. of equine lysozyme, it was demonstrated that Ca2+ binding induces a conformational change upon which hydrophobic regions in the protein become less accessible. Bis-ANS fluorescence titrns. in the absence of Ca2+ and in 2 mM Ca2+ were also performed with equine α-**lactalbumin** variants B and C. These variants differed by the amino acid exchange Asp-95 → Ile. The fluorescence titration curves indicated that the accessibility of the probe to the Ca2+ conformers was clearly influenced by the **mutation**. The Ca2+-dependent exclusion of a hydrophobic domain was used in a new and simplified method for preparing lysozyme and α- **lactalbumins** simultaneously from equine milk whey.

IT **7440-70-2, Calcium,** analysis
RL: ANST (Analytical study)
(**lactalbumin** and lysozyme of horse milk whey separation by hydrophobic interaction chromatog. in presence of)

L9 ANSWER 22 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 28 Nov 1992
ACCESSION NUMBER: 1992:607443 CAPLUS
DOCUMENT NUMBER: 117:207443
TITLE: Thermodynamic changes in the binding of **calcium** to a **mutant** human lysozyme (D86/92). Enthalpy-entropy compensation observed upon **calcium** binding to proteins
AUTHOR(S): Kuroki, Ryota; Nitta, Katsutoshi; Yutani, Katsuhide
CORPORATE SOURCE: Prot. Eng. Res. Inst., Osaka, 565, Japan
SOURCE: Journal of Biological Chemistry (1992), 267(34), 24297-301
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The thermodn. change in the binding of Ca2+ to a **mutant** human lysozyme having an engineered Ca2+ binding site (Kuroki, R., et. al., 1989) was analyzed by calorimetry and interpreted in terms of structural information obtained from x-ray crystallog. It was found that the enthalpic contribution for the Ca2+ binding reaction was small, driven primarily by entropy release (10 kcal/mol). This release of entropy was also observed in some organic chelators. Moreover, through the information of the tertiary structures of the apo- and holomutant lysozyme, it was confirmed that the entropy release (10 kcal/mol) upon the binding of Ca2+ arises primarily from the release of bound water mols. hydrating the free Ca2+. Previous studies of Ca2+ binding to proteins have involved significant changes in protein conformation. They can now be reevaluated to determine the contribution of

conformational changes to Ca2+ binding. After removing the thermodn. contribution of Ca2+ binding itself, it is found that upon the binding of Ca2+ the enthalpy change is neg. but is almost compensated by the neg. entropy change. The neg. change in both enthalpy and entropy is characteristic of values seen in the thermodn. change upon the folding of proteins.

IT **7440-70-2, Calcium,** biological studies
RL: BIOL (Biological study)
(proteins binding of, thermodn. and conformational contributions to)

L9 ANSWER 23 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 01 Nov 1991
ACCESSION NUMBER: 1991:578155 CAPLUS
DOCUMENT NUMBER: 115:178155
TITLE: Crystal structures of the apo- and holomutant human lysozymes with an introduced **calcium** binding site
AUTHOR(S): Inaka, Koji; Kuroki, Ryota; Kikuchi, Masakazu; Matsushima, Masaaki
CORPORATE SOURCE: Protein Eng. Res. Inst., Suita, 565, Japan
SOURCE: Journal of Biological Chemistry (1991), 266(31), 20666-71
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The 3-dimensional structures of **mutant** apo- and holo human lysozyme (I) (Q86D/A92D-I), in which a Ca2+-binding site was designed and created for enhancing mol. stability by replacing both Gln-86 and Ala-92 with aspartic acids, were refined at 1.8-Å resolution by x-ray crystallog. The overall structures and crystallog. thermal factors of all 3 proteins, human apo- and holo-Q86D/A92D-I, and wild-type I, were essentially identical; these results showed that the introduction of the Ca2+-binding site did not affect either the overall structure or mol. rigidity of the proteins. However, structure analyses of apo-Q86D/A92D-I revealed that the **mutations** affected the side-chain conformation of residue 86 and H-networks between the protein and the internal solvent mols. In the structure of holo-Q86D/A92D-I, 7 O ligands formed a slightly distorted pentagonal bipyramid around the Ca2+, indicating that the coordination around Ca2+ was quite similar to that in baboon α- **lactalbumin** . The pentagonal bipyramid coordination could be one of the most widely found and appropriate Ca2+ binding schemes in proteins.

IT **7440-70-2, Calcium,** biological studies
RL: BIOL (Biological study)
(lysozyme **mutant** engineered binding site for, of human, crystallog. study of)

L9 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2005 ACS on STN
ED Entered STN: 20 Feb 1988
ACCESSION NUMBER: 1988:51995 CAPLUS
DOCUMENT NUMBER: 108:51995
TITLE: Structure of the pigeon lysozyme and its relationship with other type c lysozymes
AUTHOR(S): Rodriguez, Rosalia; Menendez-Arias, Luis; Gonzalez de Buitrago, Gonzalo; Gavilanes, Jose G.
CORPORATE SOURCE: Fac. Cienc., Univ. Complutense, Madrid, 28040, Spain
SOURCE: Comparative Biochemistry and Physiology, Part B:

Biochemistry & Molecular Biology (1987), 88B(3), 791-6
CODEN: CBPBB8; ISSN: 0305-0491
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The secondary structure of the pigeon egg white lysozyme shows important differences when compared to other type c lysozymes. These differences are mainly located at the region comprising residues 77-84. This segment contains 1 α-helix in the lysozymes c studied by an x-ray anal., while the residues at such positions in pigeon lysozyme would form 2 β-bends. Anal. of the tertiary structure of the pigeon lysozyme by means of hydropathy profiles reveals that the above segment seems to be more hydrophilic in the pigeon enzyme than in other type c lysozymes. Though a certain similarity to the **calcium**-binding loop of α-**lactalbumins** is detected in pigeon lysozyme, the CD spectra of the protein at neutral pH do not change in the presence of Ca2+. The presented structural anal. is discussed in terms of function-structure and antigenicity relationships between the type c lysozymes.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CANCERLIT' ENTERED AT 14:45:58 ON 08 APR 2005)

L10 1147 SEA ABB=ON PLU=ON L6
L11 88 SEA ABB=ON PLU=ON L10 AND (MUTAT? OR MUTAGEN? OR POLYMORPH? OR POLY MORPH? OR MUTANT)
L12 16 SEA ABB=ON PLU=ON L10 AND (K79 OR D82 OR D84 OR D87# OR D88 OR S70R)
L13 88 SEA ABB=ON PLU=ON L11 OR L12
L14 41 DUP REM L13 (47 DUPLICATES REMOVED)

L14 ANSWER 1 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:874030 SCISEARCH
THE GENUINE ARTICLE: 856WT
TITLE: Protein encapsulation via porous CaCO3 microparticles templating
AUTHOR: Volodkin D V (Reprint); Larionova N I; Sukhorukov G B
CORPORATE SOURCE: Max Planck Inst Colloids & Interfaces, D-14476 Potsdam, Germany (Reprint); Moscow MV Lomonosov State Univ, Dept Chem, Moscow 119992, Russia
COUNTRY OF AUTHOR: Germany; Russia
SOURCE: BIOMACROMOLECULES, (SEP-OCT 2004) Vol. 5, No. 5, pp. 1962-1972.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
ISSN: 1525-7797.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 49

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Porous microparticles of **calcium** carbonate with an average diameter of 4.75 mum were prepared and used for protein encapsulation in polymer-filled microcapsules by means of electrostatic layer-by-layer assembly (ELbL). Loading of macromolecules in porous CaCO3 particles is affected by their molecular weight due to diffusion-limited permeation inside the particles and also by the affinity to the carbonate surface. Adsorption of various proteins and dextran was examined as a function of pH and was found to be dependent both on the charge of the

microparticles and macromolecules. The electrostatic effect was shown to govern this interaction. This paper discusses the factors which can influence the adsorption capacity of proteins. A new way of protein encapsulation in polyelectrolyte microcapsules is proposed exploiting the porous, biocompatible, and decomposable microparticles from CaCO3. It consists of protein adsorption in the pores of the microparticles followed by ELbL of oppositely charged polyelectrolytes and further core dissolution. This resulted in formation of polyelectrolyte-filled capsules with protein incorporated in interpenetrating polyelectrolyte network. The properties of CaCO3 microparticles and capsules prepared were characterized by scanning electron microscopy, microelectrophoresis, and confocal laser scanning microscopy. **Lactalbumin** was encapsulated by means of the proposed technique yielding a content of 0.6 pg protein per microcapsule. Horseradish peroxidase saves 37% of activity after encapsulation. However, the termostability of the enzyme was improved by encapsulation. The results demonstrate that porous CaCO3 microparticles can be applied as microtemplates for encapsulation of proteins into polyelectrolyte capsules at neutral pH as an optimal medium for a variety of bioactive material, which can also be encapsulated by the proposed method. Microcapsules filled with encapsulated material may find applications in the field of biotechnology, biochemistry, and medicine.

L14 ANSWER 2 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 1

ACCESSION NUMBER: 2004:420433 BIOSIS
DOCUMENT NUMBER: PREV200400421865
TITLE: Localized nature of the transition-state structure in goat alpha-**lactalbumin** folding.
AUTHOR(S): Saeki, Kimiko; Arai, Munehito; Yoda, Takao; Nakao, Masaharu; Kuwajima, Kunihiro [Reprint Author]
CORPORATE SOURCE: Grad Sch SciDept PhysBunkyo Ku, Univ Tokyo, 7-3-1 Hongo, Tokyo, 1130033, Japan
kuwajima@phys.s.u-tokyo.ac.jp
SOURCE: Journal of Molecular Biology, (August 6 2004) Vol. 341, No. 2, pp. 589-604. print.
ISSN: 0022-2836 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Nov 2004
Last Updated on STN: 3 Nov 2004

AB To investigate whether the structure partially formed in the molten globule folding intermediate of goat alpha-**lactalbumin** is further organized in the transition state of folding, we constructed a number of **mutant** proteins and performed PHI-value analysis on them. For this purpose, we measured the equilibrium unfolding transitions and kinetic refolding and unfolding reactions of the **mutants** using equilibrium and stopped-flow kinetic circular dichroism techniques. The results show that the **mutants** with **mutations** located in the A-helix (V8A, L12A), the B-helix (V27A), the beta-domain (L52A, W60A), the C-helix (K93A, L96A), the C-D loop (Y103F), the D-helix (L105A, L110A), and the C-terminal 310-helix (W118F), have low PHI-values, less than 0.2. On the other hand, **D87N**, which is located on the Ca2+-binding site, has a high PHI-value, 0.91, indicating that tight packing of the side-chain around Asp87 occurs in the transition state. One beta-domain **mutant** (I55V) and three C-helix **mutants** (I89V, V90A, and I95V) demonstrated intermediate PHI-values, between

0.4 and 0.7. These results indicate that the folding nucleus in the transition state of goat alpha-LA is not extensively distributed over the alpha-domain of the protein, but very localized in a region that contains the Ca2+-binding site and the interface between the C-helix and the beta-domain. This is apparently in contrast with the fact that the molten globule state of alpha-**lactalbumin** has a partially formed structure inside the alpha-domain. It is concluded that the specific docking of the alpha and beta-domains at a domain interface is necessary for this protein to organize its native structure from the molten globule intermediate.

L14 ANSWER 3 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2004:708710 SCISEARCH
THE GENUINE ARTICLE: 842ZG
TITLE: A non-native alpha-helix is formed in the beta-sheet region of the molten globule state of canine milk lysozyme
AUTHOR: Watanabe M; Kobashigawa Y; Aizawa T; Demura M; Nitta K (Reprint)
CORPORATE SOURCE: Hokkaido Univ, Grad Sch Sci, Div Biol Sci, Sapporo, Hokkaido 0600810, Japan (Reprint)
COUNTRY OF AUTHOR: Japan
SOURCE: PROTEIN JOURNAL, (JUL 2004) Vol. 23, No. 5, pp. 335-342.
Publisher: KLUWER ACADEMIC/PLENUM PUBL, 233 SPRING ST, NEW YORK, NY 10013 USA.
ISSN: 1572-3887.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The native and the molten globule states (N and MG states, respectively) of canine milk lysozyme (CML) were examined by CD spectroscopy and AGADIR algorithm, a helix-coil transition program. It revealed that the helical content of the MG state was higher than that of the N-state, suggesting that non-native alpha-helix is formed in the MG state of CML. Using AGADIR, it indicated the possibility of alpha-helix formation in the third beta-strand region in the MG state. To investigate this possibility, we designed a **mutant**, Q58P, in which the helical propensity of the MG state was significantly decreased around the third beta-strand region. It appeared that the absolute ellipticity value at 222 nm of the **mutant** in the MG state was smaller than that of the wild-type protein. It could be assumed that the non-native alpha-helix is formed around the third beta-strand region of wild-type CML in the MG state.

L14 ANSWER 4 OF 41 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2004092335 EMBASE
TITLE: The interaction of proteins with solid surfaces.
AUTHOR: Gray J.J.
CORPORATE SOURCE: J.J. Gray, Chem. and Biomolecular Engineering, Johns Hopkins University, 3400 North Charles Street, Baltimore, MD 21218, United States, jgray@jhu.edu
SOURCE: Current Opinion in Structural Biology, (2004) Vol. 14, No. 1, pp. 110-115.
Refs: 88

ISSN: 0959-440X CODEN: COSBEF
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040318
Last Updated on STN: 20040318

AB The interaction of proteins with solid surfaces is a fundamental phenomenon with implications for nanotechnology, biomaterials and biotechnological processes. Kinetic and thermodynamic studies have long indicated that significant conformational changes may occur as a protein encounters a surface; new techniques are measuring and modeling these changes. Combinatorial and directed evolution techniques have created new peptide sequences that bind specifically to solid surfaces, similar to the natural proteins that regulate crystal growth. Modeling efforts capture kinetics and thermodynamics on the colloidal scale, but detailed treatments of atomic structure are still in development and face the usual challenges of protein modeling. Opportunities abound for fundamental discovery, as well as breakthroughs in biomaterials, biotechnology and nanotechnology.

L14 ANSWER 5 OF 41 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2003-903973 [82] WPIDS
DOC. NO. CPI: C2003-257181
TITLE: New biologically active complex of alpha-**lactalbumin**, such as HAMLET, its biologically active modification or fragment, useful for preparing a medicament of treating papilloma.
DERWENT CLASS: B04 D13
INVENTOR(S): SVANBORG, C
PATENT ASSIGNEE(S): (SVAN-I) SVANBORG C
COUNTRY COUNT: 104
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003095490	A1	20031120	(200382)*	EN	22
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
AU 2003233116	A1	20031111	(200442)		
EP 1506233	A1	20050216	(200513)	EN	
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003095490	A1	WO 2003-IB2366	20030508
AU 2003233116	A1	AU 2003-233116	20030508
EP 1506233	A1	EP 2003-727868	20030508
		WO 2003-IB2366	20030508

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003233116	A1 Based on	WO 2003095490
EP 1506233	A1 Based on	WO 2003095490

PRIORITY APPLN. INFO: GB 2002-10464 20020508
AN 2003-903973 [82] WPIDS
AB WO2003095490 A UPAB: 20031223
NOVELTY - A biologically active complex of alpha -**lactalbumin**, such as human alpha -**lactalbumin** made lethal to tumor cells (HAMLET), its biologically active modification or fragment, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for treating a papilloma virus by administering to a patient, the biologically active complex of alpha -**lactalbumin** cited above.

ACTIVITY - Virucide. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The biologically active complex of alpha -**lactalbumin** is useful for preparing a medicament of treating papilloma (claimed), preferably cutaneous papilloma.

Dwg.0/0

L14 ANSWER 6 OF 41 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
ACCESSION NUMBER: 2003-690031 [65] WPIDS
DOC. NO. CPI: C2003-189264
TITLE: Complex of alpha-**lactalbumin** and a cofactor that stabilizes the complex in a biologically active form, useful for treating cancer and bacterial infections.
DERWENT CLASS: B04 D16
INVENTOR(S): SVANBORG, C; SVENSSON, M W
PATENT ASSIGNEE(S): (SVAN-I) SVANBORG C; (SVEN-I) SVENSSON M W
COUNTRY COUNT: 97
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003074547	A2	20030912	(200365)*	EN	56
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2003214522	A1	20030916	(200430)		
EP 1485413	A2	20041215	(200482)	EN	
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003074547	A2	WO 2003-IB1293	20030307
AU 2003214522	A1	AU 2003-214522	20030307
EP 1485413	A2	EP 2003-710101	20030307

WO 2003-IB1293 20030307

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2003214522	A1 Based on	WO 2003074547
EP 1485413	A2 Based on	WO 2003074547

PRIORITY APPLN. INFO: GB 2002-5347 20020307
AN 2003-690031 [65] WPIDS
AB WO2003074547 A UPAB: 20031009
NOVELTY - New complex comprises alpha -**lactalbumin**, or an alpha -**lactalbumin** variant which is in the apo folding state, or a fragment of either of these, and a cofactor that stabilizes the complex in a biologically active form.

DETAILED DESCRIPTION - New complex comprises alpha -**lactalbumin**, or an alpha -**lactalbumin** variant which is in the apo folding state, or a fragment of either of these, and a cofactor that stabilizes the complex in a biologically active form, provided that the fragment comprises a region corresponding to the region of alpha -**lactalbumin** that forms the interface between the alpha and beta domains, and the cofactor is not C18:1:9 cis fatty acid when the complex comprises native alpha -**lactalbumin**.

INDEPENDENT CLAIMS are also included for:

(1) a pharmaceutical composition comprising the complex and a carrier;

(2) treatment of cancer by administering the complex to cancer cells;

(3) treatment of bacterial infections by administering the complex to a patient.

ACTIVITY - Cytostatic; Antibacterial. Viability of L1210 leukemia cells after incubation with a complex of alpha -**lactalbumin** and C18:1:11 cis fatty acid was 1%, compared with 99% for untreated cells and 0% for cells incubated with HAMLET (complex of alpha -**lactalbumin** and C18:1:9 cis fatty acid).

MECHANISM OF ACTION - Apoptosis inducer.

USE - The complex is useful for treating cancer and bacterial infections.

Dwg.0/11

L14 ANSWER 7 OF 41 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003561339 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14627739
TITLE: Alpha-**lactalbumin** unfolding is not sufficient to cause apoptosis, but is required for the conversion to HAMLET (human alpha-**lactalbumin** made lethal to tumor cells).
AUTHOR: Svensson Malin; Fast Jonas; Mossberg Ann-Kristin; Duringer Caroline; Gustafsson Lotta; Hallgren Oskar; Brooks Charles L; Berliner Lawrence; Linse Sara; Svanborg Catharina
CORPORATE SOURCE: Department of Microbiology, Immunology and Glycobiology (MIG), Institute of Laboratory Medicine, Lund University, Lund, Sweden.
SOURCE: Protein science : a publication of the Protein Society, (2003 Dec) 12 (12) 2794-804.
Journal code: 9211750. ISSN: 0961-8368.

Searcher : Shears 571-272-2528

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200407
ENTRY DATE: Entered STN: 20031216
Last Updated on STN: 20040715
Entered Medline: 20040714

AB HAMLET (human alpha-**lactalbumin** made lethal to tumor cells) is a complex of human alpha-**lactalbumin** and oleic acid (C18:1:9 cis) that kills tumor cells by an apoptosis-like mechanism. Previous studies have shown that a conformational change is required to form HAMLET from alpha-**lactalbumin**, and that a partially unfolded conformation is maintained in the HAMLET complex. This study examined if unfolding of alpha-**lactalbumin** is sufficient to induce cell death. We used the bovine alpha-**lactalbumin** Ca(2+) site **mutant D87A**, which is unable to bind Ca(2+), and thus remains partially unfolded regardless of solvent conditions. The **D87A mutant** protein was found to be inactive in the apoptosis assay, but could readily be converted to a HAMLET-like complex in the presence of oleic acid. BAMLET (bovine alpha-**lactalbumin** made lethal to tumor cells) and **D87A**-BAMLET complexes were both able to kill tumor cells. This activity was independent of the Ca(2+)site, as HAMLET maintained a high affinity for Ca(2+) but **D87A**-BAMLET was active with no Ca(2+) bound. We conclude that partial unfolding of alpha-**lactalbumin** is necessary but not sufficient to trigger cell death, and that the activity of HAMLET is defined both by the protein and the lipid cofactor. Furthermore, a functional Ca(2+)-binding site is not required for conversion of alpha-**lactalbumin** to the active complex or to cause cell death. This suggests that the lipid cofactor stabilizes the altered fold without interfering with the Ca(2+)site.

L14 ANSWER 8 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2003:160805 SCISEARCH
THE GENUINE ARTICLE: 643RN
TITLE: Recoverin is a zinc-binding protein
AUTHOR: Permyakov S E; Cherskaya A M; Wasserman L A; Khokhlova T I; Senin I I; Zargarov A A; Zinchenko D V; Zernii E Y; Lipkin V M; Philippov P P; Uversky V N (Reprint); Permyakov E A
CORPORATE SOURCE: Russian Acad Sci, Inst Biol Instrumentat, Pushchino 142290, Moscow Region, Russia (Reprint); Russian Acad Sci, Inst Biochem Phys, Moscow 117334, Russia; Univ Calif Santa Cruz, Dept Chem & Biochem, Santa Cruz, CA 95064 USA; M M Shemyakin & Y A Ovchinnikov Inst Bioorgan Che, Branch, Pushchino 142290, Moscow Region, Russia; Moscow MV Lomonosov State Univ, A N Belozersky Inst Physicochem Biol, Dept Cell Signaling, Moscow 119899, Russia
COUNTRY OF AUTHOR: Russia; USA
SOURCE: JOURNAL OF PROTEOME RESEARCH, (JAN-FEB 2003) Vol. 2, No. 1, pp. 51-57.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
ISSN: 1535-3893.
DOCUMENT TYPE: Article; Journal

LANGUAGE: English
REFERENCE COUNT: 45
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recoverin is an N-myristoylated 23 kDa **calcium**-binding protein from retina, which modulates the Ca2+-sensitive deactivation of rhodopsin via Ca2+-dependent inhibition of rhodopsin kinase. It was shown by intrinsic and bis-ANS probe fluorescence, circular dichroism, and differential scanning calorimetry that myristoylated recombinant recoverin interacts specifically with zinc ions. Similar to the **calcium** binding, the binding of zinc to Ca2+-loaded recoverin additionally increases its a-helical content, hydrophobic surface area, and environmental mobility/polarity of its tryptophan residues. In contrast to the **calcium** binding, the binding of zinc decreases thermal stability of the Ca2+-loaded protein. Zn2+-titration of recoverin, traced by bis-ANS fluorescence, reveals binding of a single Zn2+ ion per protein molecule. It was shown that the double-**mutant** E85Q/E121Q with inactivated Ca2+-binding EF-hands 2 and 3 (Alekseev, A. M.; Shulga-Morskoy, S. V.; Zinchenko, D. V.; Shulga-Morskaya, S. A.; Suchkov, D. V.; Vaganova, S. A.; Senin, I. I.; Zargarov, A. A.; Lipkin, V. M.; Akhtar, M.; Philippov, P. P. FEBS Lett. 1998, 440, 116-118), which can be considered as an analogue of the apo-protein, binds Zn2+ ion as well. Apparent zinc equilibrium binding constants evaluated from spectrofluorimetric Zn2+-titrations of the protein are 1.4 x 10(5) M-1 (dissociation constant 7.1 muM) for Ca2+-loaded wild-type recoverin and 3.3 x 10(4) M-1 (dissociation constant 30 muM) for the E85Q/E121Q **mutant** (analogue of apo-recoverin). Study of the binding of wild-type recoverin to ROS membranes showed a zinc-dependent increase of its affinity for the membranes, without regard to **calcium** content, suggesting further solvation of a protein myristoyl group upon Zn2+ binding. Possible implications of these findings to the functioning of recoverin are discussed.

L14 ANSWER 9 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 3

ACCESSION NUMBER: 2002:462273 BIOSIS
DOCUMENT NUMBER: PREV200200462273
TITLE: Studies on the metal binding sites in the catalytic domain of beta1,4-galactosyltransferase.
AUTHOR(S): Boeggeman, Elizabeth; Qasba, Pradman K. [Reprint author]
CORPORATE SOURCE: Structural Glycobiology Section, Laboratory of Experimental and Computational Biology, NCI-CCR, Building 469, Room 221, Frederick, MD, 21702-1201, USA qasba@helix.nih.gov
SOURCE: Glycobiology, (July, 2002) Vol. 12, No. 7, pp. 395-407. print.
ISSN: 0959-6658.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Aug 2002
Last Updated on STN: 28 Aug 2002

AB The catalytic domain of bovine beta1,4-galactosyltransferase (beta4Gal-T1) has been shown to have two metal binding sites, each with a distinct binding affinity. Site I binds Mn2+ with high affinity and does not bind Ca2+, whereas site II binds a variety of metal ions, including Ca2+. The catalytic region of beta4Gal-T1 has DXD motifs, associated with metal binding in glycosyltransferases, in two separate sequences: D242YDYNCFVFSDVD254 (region I) and

W312GWGGEDDD320 (region II). Recently, the crystal structure of beta4Gal-T1 bound with UDP, Mn2+, and alpha-**lactalbumin** was determined in our laboratory. It shows that in the primary metal binding site of beta4Gal-T1, the Mn2+ ion, is coordinated to five ligands, two supplied by the phosphates of the sugar nucleotide and the other three by Asp254, His347, and Met344. The residue Asp254 in the D252VD254 sequence in region I is the only residue that is coordinated to the Mn2+ ion. Region II forms a loop structure and contains the E317DDD320 sequence in which residues Asp318 and Asp319 are directly involved in GlcNAc binding. This study, using site-directed **mutagenesis**, kinetic, and binding affinity analysis, shows that Asp254 and His347 are strong metal ligands, whereas Met344, which coordinates less strongly, can be substituted by alanine or glutamine. Specifically, substitution of Met344 to Gln has a less severe effect on the catalysis driven by Co2+. Glu317 and Asp320 **mutants**, when partially activated by Mn2+ binding to the primary site, can be further activated by Co2+ or inhibited by Ca2+, an effect that is the opposite of what is observed with the wild-type enzyme.

L14 ANSWER 10 OF 41 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2002293634 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11979521
TITLE: A metal binding in the polypeptide chain improves the folding efficiency of a denatured and reduced protein.
AUTHOR: Ohkuri Takatoshi; Ueda Tadashi; Yoshida Yuichiro; Abe Yoshito; Hamasaki Naotaka; Imoto Taiji
CORPORATE SOURCE: Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan.
SOURCE: Biopolymers, (2002 Jul 5) 64 (2) 106-14.
Journal code: 0372525. ISSN: 0006-3525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200207
ENTRY DATE: Entered STN: 20020530
Last Updated on STN: 20020703
Entered Medline: 20020702

AB In order to examine the effect of a metal binding to the polypeptide chain on the aggregation of a protein in the refolding process, we prepared a **mutant** hen lysozyme possessing the same Ca(2+) binding site as in human alpha-**lactalbumin** by Escherichia coli expression system (Ser(-1) CaB lysozyme). In the presence of 2 mM CaCl(2), the refolding yield of Ser(-1) CaB lysozyme at a low protein concentration (25 microg/mL) was similar to that of the wild-type lysozyme (80%), but that at high protein concentration (200 microg/mL) decreased (15%) due to aggregation comparing to that of the wild-type lysozyme (45%). However, the refolding yield of Ser(-1) CaB lysozyme in the presence of 100 mM CaCl(2) even at a protein concentration of 200 microg/mL was 80% and was higher than that of the wild-type lysozyme. From analysis of chemical shift changes of the cross peaks in the backbone region of total correlated spectroscopy (TOCSY) spectra of a decapeptide possessing the same **calcium** binding site as in Ser(-1) CaB lysozyme in the presence of various concentrations of **Ca**(2+), it was suggested that the dissociation constant of **Ca**(2+)-peptide complex was estimated to be 20-36 mM. Moreover, the solubility of the denatured Ser(-1) CaB lysozyme in the presence of 100 mM CaCl(2) was higher than

that in the presence of 2 mM CaCl(2) whereas the solubility of the denatured Ser(-1) lysozyme in the presence of 100 mM CaCl(2) was not higher than that in the presence of 2 mM CaCl(2). Therefore, it was concluded that the reduced lysozyme possessing the Ca(2+) binding site was efficiently folded in the presence of high concentration of Ca(2+) (100 mM) even at high protein concentration due to depression of aggregation by the binding of Ca(2+) to the polypeptide chain in Ser(-1) CaB lysozyme.

L14 ANSWER 11 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:702964 SCISEARCH
THE GENUINE ARTICLE: 467YT
TITLE: Cation-dependent stability of subtilisin
AUTHOR: Alexander P A; Ruan B; Bryan P N (Reprint)
CORPORATE SOURCE: Univ Maryland, Inst Biotechnol, Ctr Adv Res Biotechnol, 9600 Gudelsky Dr, Rockville, MD 20850 USA (Reprint); Univ Maryland, Inst Biotechnol, Ctr Adv Res Biotechnol, Rockville, MD 20850 USA
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (4 SEP 2001) Vol. 40, No. 35, pp. 10634-10639.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 33
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Subtilisin BPN ' contains two cation binding sites. One specifically binds **calcium** (site A), and the other can bind both divalent and monovalvent metals (site B). By binding at specific sites in the tertiary structure of subtilisin, cations contribute their binding energy to the stability of the native state and increase the activation energy of unfolding. Deconvoluting the influence of binding sites A and B on the inactivation rate of subtilisin is complicated, however. This paper examines the stabilizing effects of cation binding at site B by using a **mutant** of subtilisin BPN ' which lacks **calcium** site A. Using this **mutant**, we show that **calcium** binding at site B has relatively little effect on stability in the presence of moderate concentrations of monovalent cations. At [NaCl] = 100 mM, site B is greater than or equal to 98% occupied with sodium, and therefore its net occupancy with a cation varies little as subtilisin is titrated with **calcium**. Exchanging sodium for **calcium** results in a 5-fold decrease in the rate of inactivation. In contrast, because of the high selectivity of site A for **calcium**, its occupancy changes dramatically as **calcium** concentration is varied, and consequently the inactivation rate of subtilisin decreases similar to 200-fold as site A becomes saturated with **calcium**, irrespective of the concentration of monovalent cations.

L14 ANSWER 12 OF 41 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002083565 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11809927
TITLE: Energetics of three-state unfolding of a protein: canine milk lysozyme.
AUTHOR: Koshiba T; Kobashigawa Y; Demura M; Nitta K

CORPORATE SOURCE: Division of Biological Sciences, Graduate School of Science, Hokkaido University, Kita-ku, Sapporo 060-0810, Japan.
SOURCE: Protein engineering, (2001 Dec) 14 (12) 967-74.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020128
Last Updated on STN: 20020813
Entered Medline: 20020812

AB Thermodynamics of thermal transitions of a **calcium**-binding lysozyme, canine milk lysozyme (CML), was studied using differential scanning calorimetry and compared with those for homologous proteins, human alpha-**lactalbumin** (alpha-hLA) and equine milk lysozyme (EML). The results showed that CML and EML exhibit two clear heat absorption peaks in the absence of **calcium** ions (apo-form), although the cooperative thermal transition of alpha-hLA is apparently absent in this form. The first peak represents the unfolding transition from the native to an unfolding intermediate state (N-I transition) and the second peak represents that from the intermediate to the thermally unfolded state (I-U transition). We interpret that the cooperative thermal transition, which is observed between the intermediate and the thermally unfolded states of CML and EML, comes from the native-like packing interaction in their intermediate states. Furthermore, to examine the role of the stabilization mechanism of CML intermediate, we constructed four variant CMLs (H21G, I56L, A93S and V109K), in which the residues of CML are substituted for those of EML, and also investigated their thermal stability. Especially the His21 and Val109 of CML play a role in stabilization of the intermediate state and their contributions to the unfolding free energy are estimated to be 2.0 and 1.8 kJ/mol, respectively. From the results of the **mutational** analysis, a few differences in the local helical interactions within the alpha-domain are found to be predominant in stabilizing the intermediate state.

L14 ANSWER 13 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:556997 BIOSIS
DOCUMENT NUMBER: PREV200100556997
TITLE: Metal binding studies of the catalytic domain of bovine beta-1,4-galactosyltransferase.
AUTHOR(S): Boeggeman, Elizabeth [Reprint author]; Qasba, Pradman K. [Reprint author]
CORPORATE SOURCE: Structural Glycobiology Section, LECB, NCI-CCR, Bldg 469, Room 221, Frederick, MD, 21702-1201, USA
SOURCE: Glycobiology, (October, 2001) Vol. 11, No. 10, pp. 924. print.
Meeting Info.: 6th Annual Conference of the Society for Glycobiology. San Francisco, California, USA. November 14-17, 2001.
ISSN: 0959-6658.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Dec 2001
Last Updated on STN: 25 Feb 2002

Searcher : Shears 571-272-2528

L14 ANSWER 14 OF 41 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 2002096539 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11739897
TITLE: **Mutating** aspartate in the **calcium** -binding site of alpha-**lactalbumin**: effects on the protein stability and cation binding.
AUTHOR: Permyakov S E; Uversky V N; Veprintsev D B; Cherskaya A M; Brooks C L; Permyakov E A; Berliner L J
CORPORATE SOURCE: Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino, Moscow region 142290, Russia.
CONTRACT NUMBER: GM 56970 (NIGMS)
SOURCE: Protein engineering, (2001 Oct) 14 (10) 785-9. Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200203
ENTRY DATE: Entered STN: 20020206
Last Updated on STN: 20020319
Entered Medline: 20020318

AB The residue Asp87, which is in the **calcium**-binding loop of bovine alpha-**lactalbumin** (alpha-LA) and provides a side-chain carboxylate oxygen for ligand **Ca**(II) co-ordination, was substituted by either alanine or asparagine. The physical properties and **calcium**-binding affinities were monitored by intrinsic fluorescence and circular dichroism spectroscopy. **D87A** alpha-LA displayed a total loss of rigid tertiary structure, a dramatic loss in secondary structure and negligible **calcium** affinity [Anderson et al. (1997) Biochemistry, 36, 11648-11654]. On the contrary, **D87N** alpha-LA displayed native-like secondary structure with a somewhat de-stabilized tertiary structure. When the well-documented N-terminal methionine was enzymatically removed from **D87N** alpha-LA [Veprintsev et al. (1999) PROTEINS: Struct. Funct. Genet., 37, 65-72], the structure appeared to more closely resemble native alpha-LA. Remarkably, the thermal transition mid-temperature of apo-desMetD87N alpha-LA was approximately 31 degrees C versus native apo- alpha-LA (approximately 25 degrees C), probably due to negative charge 'compensation' in the **calcium** co-ordination site. On the other hand, the transition mid-temperature of Ca(II)-bound desMetD87N alpha-LA was approximately 57 degrees C versus native alpha-LA (approximately 66 degrees C), which was related to a decreased Ca(II) affinity (K = approximately 2.1 x 10(5) versus approximately 1.7 x 10(7)/M at 40 degrees C, respectively). These results reaffirm that alanine substitution in site specific **mutagenesis** is not always a prudent choice. Substitutions must be conservative with only minimal changes in functional groups and side-chain volume.

L14 ANSWER 15 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:789461 SCISEARCH
THE GENUINE ARTICLE: 477UA
TITLE: Oxidative folding of human lysozyme: Effects of the loss of two disulfide bonds and the introduction of a **calcium**-binding site

AUTHOR: Kurokawa Y; Koganesawa N; Kobashigawa Y; Koshiba T; Demura M; Nitta K (Reprint)
CORPORATE SOURCE: Hokkaido Univ, Grad Sch Sci, Div Biol Sci, Sapporo, Hokkaido 0600810, Japan (Reprint)
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF PROTEIN CHEMISTRY, (MAY 2001) Vol. 20, No. 4, pp. 293-303.
Publisher: KLUWER ACADEMIC/PLENUM PUBL, 233 SPRING ST, NEW YORK, NY 10013 USA.
ISSN: 0277-8033.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 35
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Mutant** human lysozymes (HLZ) lacking two disulfide bonds were constructed to study the importance of each disulfide bond on oxidative refolding. To avoid destabilization, a **calcium** -binding site was introduced. Five of the six species of two-disulfide **mutants** could be obtained with enzymatic activity. Based on the information obtained from refolding and unfolding experiments, the order of importance in oxidative refolding was found to be as follows: SS2(Cys30-Cys116) > SS1(Cys6-Cys128) approximate to SS3(Cys65-Cys81) > SS4(Cys77-Cys95). Without SS2, these **mutants** refolded with low efficiency or did not refold at all. The bond SS2 is located in the interface of B-and D-helices, and a small hydrophobic cluster is formed near SS2. This cluster may play an important role in the folding process and stabilization, and SS2 may act as a stabilizer through its polypeptide linkage. The bond SS2 is the most important disulfide bond for oxidative folding of lysozymes.

L14 ANSWER 16 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2001:26554 SCISEARCH
THE GENUINE ARTICLE: 386TG
TITLE: alpha-**Lactalbumin mutant** acting as lysozyme
AUTHOR: Xue Y M; Liu J N; Sun Z Y; Ma Z; Wu C L; Zhu D X (Reprint)
CORPORATE SOURCE: Nanjing Univ, Dept Biochem, State Key Lab Pharmaceut Biotechnol, 22 Hankou Rd, Nanjing, Peoples R China (Reprint); Nanjing Univ, Dept Biochem, State Key Lab Pharmaceut Biotechnol, Nanjing, Peoples R China; Nanjing Univ, Inst Mol Med, Nanjing 210008, Peoples R China
COUNTRY OF AUTHOR: Peoples R China
SOURCE: PROTEINS-STRUCTURE FUNCTION AND GENETICS, (1 JAN 2001) Vol. 42, No. 1, pp. 17-22.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA.
ISSN: 0887-3585.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 17
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A **mutant** of alpha -**lactalbumin** was expressed and purified, in which His32, Thr33, Glu49, Ike59, Val99, and Tyr103 were substituted by Leu32, Glu33, Asp49, Trp59, ksn99, and Ala103, respectively, to create a catalytic site of lysozyme in alpha -**lactalbumin**. The **mutant** catalyzed hydrolysis of the

synthetic substrate, pNP-(NAcGlc)(3), with a K-M and k(cat) of 0.160 +/- 0.00986 mmol/L and 3.39 +/- 0.0456 x 10(-5) min(-1), respectively, which was comparable with those of chicken lysozyme of 0.137 +/- 0.0153 mmol/L and 5.25 +/- 0.115 x 10(-4) min(-1). By using the Isothermal Titration Calorimetre (ITC), the average binding enthalpy of the **mutant** or chicken lysozyme with the substrate (chitopentaose) was measured, which was 49.22 KJ/mol for the **mutant** and 105.47 KJ/mol for chicken lysozyme. In conclusion, the six point **mutations** occurring in alpha - **lactalbumin** could be converted into an enzyme that was 17.5-fold less efficient than chicken lysozyme but nevertheless capable of hydrolyzing the glycosidic bond. Proteins 2001;42:17-22. (C) 2000 Wiley-Liss, Inc.

L14 ANSWER 17 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 7

ACCESSION NUMBER: 2001:313724 BIOSIS
DOCUMENT NUMBER: PREV200100313724
TITLE: Structural basis for the appearance of a molten globule state in chimeric molecules derived from lysozyme and alpha-**lactalbumin**.
AUTHOR(S): Joniau, Marcel; Haezebrouck, Petra; Noyelle, Katrien; Van Dael, Herman [Reprint author]
CORPORATE SOURCE: Interdisciplinary Research Centre, K.U. Leuven, Campus Kortrijk, B-8500, Kortrijk, Belgium
Herman.vandael@kulak.ac.be
SOURCE: Proteins, (July 1, 2001) Vol. 44, No. 1, pp. 1-11. print.
CODEN: PSFGEY. ISSN: 0887-3585.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Jul 2001
Last Updated on STN: 19 Feb 2002

AB The problem as to why alpha-**lactalbumin**, in the absence of Ca2+, forms a molten globule intermediate, in contrast to its structural homologue lysozyme, has been addressed by the construction of chimeras of human lysozyme in which either the Ca2+-binding loop or a part of helix C of bovine alpha-**lactalbumin** were transplanted. Previously, we have shown that the introduction of both structural elements together in the lysozyme matrix causes the apo form of the resulting chimera to display molten globule behavior during the course of thermal denaturation. In this article, we demonstrate that this molten globule character is not correlated with the Ca2+-binding loop. Also, the Del 101 **mutant** in which Arg101 was deleted to simulate the alpha-**lactalbumin** conformation of the connecting loop between helix C and helix D, does not show a stable equilibrium intermediate. Rather, the molten globule character of the chimeras has to be related with a specific part of helix C. More particularly, attention is drawn to the four hydrophobic side-chains I93, V96, I99, and L100, the lysozyme counterparts of which are constituted of less bulky valines and alanine. Our observations are discussed in terms of decreased stability of the native form and increased stability of the intermediate molten globule.

L14 ANSWER 18 OF 41 MEDLINE on STN DUPLICATE 8

ACCESSION NUMBER: 2001096296 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11155249
TITLE: [Use of method of protein engineering in studying

calcium-binding proteins].
Issledovanie metodov belkovoi inzhenerii v issledovanii kal'tsiisvaiushchikh belkov.
AUTHOR: Permiakov S E; Permiakov E A
CORPORATE SOURCE: Institute of Biological Instrumentation, Russian Academy of Sciences, Pushchino, Moscow Region, 142290 Russia.
SOURCE: Biofizika, (2000 Nov-Dec) 45 (6) 990-1006. Ref: 85
Journal code: 0372666. ISSN: 0006-3029.
PUB. COUNTRY: Russia: Russian Federation
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010201

AB Major results of the use of protein engineering methods in studies of **calcium**-binding proteins with the highest affinity for **calcium** and known three-dimensional structure (parvalbumin, calmodulin, troponin C, calbindin, recoverin, alpha-**lactalbumin**, and others) are presented. Specific features of recombinant **calcium**-binding proteins are discussed. Experiments with genetic introduction of fluorescent probes, tryptophan and tyrosine, into proteins are overviewed. Effects of **mutations** in different parts of protein molecules (**calcium**-binding loops, hydrophobic core, and others) on their structure and properties and attempts of creation of artificial **calcium**-binding sites are discussed.

L14 ANSWER 19 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:766343 SCISEARCH
THE GENUINE ARTICLE: 361DQ
TITLE: Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle - effect of milk protein **polymorphism** and different feeding regimes
AUTHOR: Devold T G (Reprint); Brovold M J; Langsrud T; Vegarud G E
CORPORATE SOURCE: AGR UNIV NORWAY, DEPT FOOD SCI, POB 5036, N-1432 AS, NORWAY (Reprint)
COUNTRY OF AUTHOR: NORWAY
SOURCE: INTERNATIONAL DAIRY JOURNAL, (JAN 2000) Vol. 10, No. 5-6, pp. 313-323.
Publisher: ELSEVIER SCI LTD, THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, OXON, ENGLAND.
ISSN: 0958-6946.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: AGRI
LANGUAGE: English
REFERENCE COUNT: 57
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Milk samples of 59 cows of the Norwegian Red Cattle breed receiving three different supplementary concentrates, were analysed for genotypes of caseins and whey proteins, the content of different milk salts (Ca2+, **Ca**, Mg and citrate), the content of total

protein, casein and whey protein and the mean micellar size of native and heated casein micelles. The genotype of alpha(s1)-casein had a statistically significant effect on the content of protein and casein, and the content of whey protein and the casein number were significantly influenced by different feeding regimes, and the content of citrate. The mean size of native and heated casein micelles was significantly influenced by the feeding regimes, genotype of alpha(s1)-casein (native mean size only) and kappa-casein, pH and the content of casein, whey protein and casein number. The heat-induced changes in mean micellar size were significantly affected by the **calcium** ion activity which accounted for approximately 40% of the total variation.

L14 ANSWER 20 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 9

ACCESSION NUMBER: 2000:304812 BIOSIS
DOCUMENT NUMBER: PREV200000304812
TITLE: alpha-**lactalbumin**: Structure and function.
AUTHOR(S): Permyakov, Eugene A. [Reprint author]; Berliner, Lawrence J.
CORPORATE SOURCE: Institute for Biological Instrumentation, Russian Academy of Sciences, Pushchino, 142292, Moscow region, Russia
SOURCE: FEBS Letters, (May 19, 2000) Vol. 473, No. 3, pp. 269-274. print.
CODEN: FEBLAL. ISSN: 0014-5793.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 19 Jul 2000
Last Updated on STN: 7 Jan 2002

AB Small milk protein alpha-**lactalbumin** (alpha-LA), a component of lactose synthase, is a simple model Ca2+ binding protein, which does not belong to the EF-hand proteins, and a classical example of molten globule state. It has a strong Ca2+ binding site, which binds Mg2+, Mn2+, Na+, and K+, and several distinct Zn2+ binding sites. The binding of cations to the Ca2+ site increases protein stability against action of heat and various denaturing agents, while the binding of Zn2+ to the Ca2+-loaded protein decreases its stability. Functioning of alpha-LA requires its interactions with membranes, proteins, peptides and low molecular weight substrates and products. It was shown that these interactions are modulated by the binding of metal cations. Recently it was found that some folding variants of alpha-LA demonstrate bactericidal activity and some of them cause apoptosis of tumor cells.

L14 ANSWER 21 OF 41 MEDLINE on STN DUPLICATE 10

ACCESSION NUMBER: 2000189636 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10727102
TITLE: Peptide analogs from E-cadherin with different **calcium**-binding affinities.
AUTHOR: Yang W; Tsai T; Kats M; Yang J J
CORPORATE SOURCE: Department of Biology, Georgia State University, Atlanta, USA.
SOURCE: journal of peptide research : official journal of the American Peptide Society, (2000 Mar) 55 (3) 203-15.
Journal code: 9707067. ISSN: 1397-002X.
PUB. COUNTRY: Denmark

Searcher : Shears 571-272-2528

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000428

AB Cadherins are a family of **calcium**-dependent cell-surface proteins that are fundamental in controlling the development and maintenance of tissues. Motif B of E-cadherin seems to be a crucial **calcium**-binding site as single point **mutations** (D134A and D134K) completely inactivate its adhesion activity. We analyzed peptide models corresponding to motif B (amino acids 128-144) as well as selected **mutations** of this motif. Our NMR studies showed that this motif B sequence is actually an active **calcium**-binding region, even in the absence of the rest of the cadherin molecule. We found that the binding affinity of this motif is very sensitive to **mutations**. For example, our peptide P128-144 with the native **calcium**-binding sequence has an affinity of Kd 0.4 mM, whereas the **mutants** P128-144/ D134A and P128-144/D134K containing the replacement of Asp134 by Ala and Lys, have Kd values of only 1.5 and 11 mM, respectively. Removing Asp at position 134, which correlates with the loss of adhesion activity, decreases **calcium**-binding affinity 20-fold. Ala132, along with residues Asp134, Asp136 and Asn143, is involved in **calcium** binding in solution. We also demonstrated that the **calcium**-binding affinity can be increased 3-fold when an additional Asp is introduced at position 132. In 50% organic solvent, this binding affinity of peptide P128-144/A132D (17-mer) from E-cadherin is similar to that of peptide P72-100/C73-77-91A (29-mer) from alpha-**lactalbumin**.

L14 ANSWER 22 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 2000:288126 SCISEARCH
THE GENUINE ARTICLE: 302PL
TITLE: Point amino acid substitutions in the Ca2+-binding sites of recoverin. II. The unusual behavior of the protein upon the binding of **calcium** ions
AUTHOR: Uversky V N; Permyakov S E; Senin I I; Cherskaya A M; ShulgaMorskoy S V; Zinchenko D V; Alekseev A M; Zargarov A A; Lipkin V M; Philippov P P; Permyakov E A (Reprint)
CORPORATE SOURCE: RUSSIAN ACAD SCI, INST BIOL INSTRUMENT MAKING, PUSHCHINO 142292, MOSCOW OBLAST, RUSSIA (Reprint); RUSSIAN ACAD SCI, INST BIOL INSTRUMENT MAKING, PUSHCHINO 142292, MOSCOW OBLAST, RUSSIA; MOSCOW MV LOMONOSOV STATE UNIV, BELOZERSKY INST PHYSICOCHEM BIOL, MOSCOW 119899, RUSSIA; RUSSIAN ACAD SCI, SHEMYAKIN OVCHINNIKOV INST BIOORGAN CHEM, PUSHCHINO BRANCH, PUSHCHINO 142292, RUSSIA
COUNTRY OF AUTHOR: RUSSIA
SOURCE: BIOORGANICHESKAYA KHIMIYA, (MAR 2000) Vol. 26, No. 3, pp. 173-178.
Publisher: MEZHDUNARODNAYA KNIGA, 39 DIMITROVA UL., 113095 MOSCOW, RUSSIA.
ISSN: 0132-3423.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE

LANGUAGE: Russian
REFERENCE COUNT: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The structural properties of myristoylated forms of recombinant recoverin of the wild type and of its **mutants** with damaged second and/or third Ca2+-binding sites were studied by fluorimetry and circular dichroism. The interaction of wild-type recoverin with **calcium** ions was shown to induce unusual structural rearrangements in its molecule. In particular, protein binding with Ca2+ ions results in an increase in the mobility of the environment of Trp residues, in higher hydrophobicity, and in elevated thermal stability (its thermal transition shifts by 15 degrees C to higher temperatures) but has almost no effect on its secondary structure. Similar structural changes induced by Ca2+ are also characteristic of the -EF2 **mutant** of recoverin whose second Ca2+-binding site is modified and cannot bind **calcium** ions. The structural properties of the -EF3 and -EF2,3 **mutants** (whose third or simultaneously second and third Ca2+-binding sites, respectively, are modified and damaged) are practically indifferent to **calcium** ions.

L14 ANSWER 23 OF 41 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 2000406865 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10813835
TITLE: Zinc binding in bovine alpha-**lactalbumin**: sequence homology may not be a predictor of subtle functional features.
AUTHOR: Permyakov S E; Veprintsev D B; Brooks C L; Permyakov E A; Berliner L J
CORPORATE SOURCE: Institute for Biological Instrumentation, Russian Academy of Science, Pushchino, Russia.
SOURCE: Proteins, (2000 Jul 1) 40 (1) 106-11.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000901
Last Updated on STN: 20000901
Entered Medline: 20000822

AB alpha-**Lactalbumin** (alpha-LA), a **calcium**-binding protein, also possesses zinc-binding sites comprising a single strong site and several weaker secondary sites. The only site found by X-ray crystallography (Ren et. al., J. Biol. Chemical 1993;268:19292) was Glu 49 of human alpha-LA, but zinc binding had never been measured in solution for human alpha-LA. This residue was genetically substituted by Ala in bovine alpha-LA and the metal-binding properties of the resulting desMetE49A protein were compared with those for native alpha-LA by fluorescence methods. Surprisingly, desMetE49A alpha-LA and the native bovine protein had similar affinities for both Zn(2+) and Ca(2+). Genetic substitution of other possible candidates for Zn(2+) chelating residues, which included Glu 25, did not alter the affinity of bovine alpha-LA to Zn2+; however, substitution of Glu 1 by Met resulted in the disappearance of strong Zn(2+) binding. A proposed site involves Glu 1, Glu 7, Asp 11, and Asp 37, which would participate in strong Zn(2+) binding based on their propinquity to Glu 1. Human alpha-LA, which has a Lys at position 1 rather than Glu, binds zinc with a reduced affinity compared with native bovine

alpha-LA, suggesting that the site identified from the X-ray structure did not correspond to strong zinc binding in solution.

L14 ANSWER 24 OF 41 WPIDS
ACCESSION NUMBER: 1999-357815 [30] WPIDS
DOC. NO. CPI: C1999-105891
TITLE: Production of oligomeric alpha-**lactalbumin** useful for inducing apoptosis in tumor cells.
DERWENT CLASS: B04 D16
INVENTOR(S): HAKANSSON, P A; SVANBORG, C; SVENSSON, M W
PATENT ASSIGNEE(S): (HAKA-I) HAKANSSON P A; (SVAN-I) SVANBORG C; (SVEN-I) SVENSSON M W
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9926979	A1	19990603	(199930)*	EN	48
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9912541	A	19990615	(199944)		
EP 1032596	A1	20000906	(200044)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
JP 2001524491	W	20011204	(200203)		53

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9926979	A1	WO 1998-IB1919	19981123
AU 9912541	A	AU 1999-12541	19981123
EP 1032596	A1	EP 1998-955823	19981123
		WO 1998-IB1919	19981123
JP 2001524491	W	WO 1998-IB1919	19981123
		JP 2000-522135	19981123

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9912541	A	Based on	WO 9926979
EP 1032596	A1	Based on	WO 9926979
JP 2001524491	W	Based on	WO 9926979

PRIORITY APPLN. INFO: GB 1998-12202 19980605; GB 1997-24725 19971121
AN 1999-357815 [30] WPIDS
AB WO 9926979 A UPAB: 19990802
NOVELTY - A new method (M1) of producing a biologically active oligomeric form of alpha -**lactalbumin** (aLA) comprises oligomerising and stabilizing aLA in the molten globule-like state,
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) a method for producing an oligomeric form of aLA which

comprises exposing a source of aLA to an ion exchange medium which has been pre-treated with casein or an active component and recovering aLA in an oligomeric form;

(2) an ion exchange medium for use in the above methods, where the medium has been treated with casein or its active components;

(3) an ion exchange column comprising the ion exchange medium of (2); and

(4) an oligomeric form of aLA obtained by a method as in (M1) or (1).

USE - The oligomeric aLA is able to induce apoptosis in tumor cells and/or has a bactericidal effect not seen with monomeric aLA. Dwg.0/8

L14 ANSWER 25 OF 41 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 1999382452 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10451551
TITLE: Fine tuning the N-terminus of a **calcium** binding protein: alpha-**lactalbumin**.
AUTHOR: Veprintsev D B; Narayan M; Permyakov S E; Uversky V N; Brooks C L; Cherskaya A M; Permyakov E A; Berliner L J
CORPORATE SOURCE: Institute for Biological Instrumentation, Russian Academy of Science, Pushchino, Russia.
SOURCE: Proteins, (1999 Oct 1) 37 (1) 65-72.
Journal code: 8700181. ISSN: 0887-3585.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199910
ENTRY DATE: Entered STN: 19991026
Last Updated on STN: 19991026
Entered Medline: 19991012

AB The effects of amino acid substitutions in the N-terminus of bovine recombinant alpha-**lactalbumin** (including enzymatic removal of the N-terminal methionine and deletion of Glu-1) were studied by intrinsic fluorescence, circular dichroism (CD), and differential scanning microcalorimetry (DSC). Wild-type recombinant alpha-**lactalbumin** has a lower thermostability and **calcium** affinity compared to the native protein, while the properties of wild-type protein with the N-terminal methionine enzymatically removed are similar to the native protein. Taken together, the fluorescence, CD, and DSC results show that recombinant wild type alpha-**lactalbumin** in the absence of **calcium** ion is in a type of molten globule state. The delta-E1 **mutant**, where the Glu(1)residue of the native sequence is genetically removed, leaving an N-terminal methionine in its place, shows almost one order of magnitude higher affinity for **calcium** and higher thermostability (both in the absence and presence of **calcium**) than the native protein isolated from milk. It was concluded that the N-terminus of the protein dramatically affects both stability and function as manifested in **calcium** affinity. Proteins 1999;37:65-72.

L14 ANSWER 26 OF 41 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 1999069427 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9852096
TITLE: Structural and thermodynamic responses of **mutations** at a Ca2+ binding site engineered

into human lysozyme.
AUTHOR: Kuroki R; Yutani K
CORPORATE SOURCE: Central Laboratories for Key Technology, Kirin Brewery Co. Ltd., 1-13-5 Fukuura, Kanazawa-ku, Yokohama 236 Japan.. r-kuroki@kirin.co.jp
SOURCE: Journal of biological chemistry, (1998 Dec 18) 273 (51) 34310-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990209
Last Updated on STN: 19990209
Entered Medline: 19990126

AB Structural determinants of Ca2+ binding sites within proteins typically comprise several acidic residues in appropriate juxtaposition. Three residues (Ala-83, Gln-86, and Ala-92) in human lysozyme are characteristically **mutated** to Lys, Asp, and Asp, respectively, in natural Ca2+ binding lysozymes and alpha-**lactalbumins**. The effects of these **mutations** on the stability and Ca2+ binding properties of human lysozyme were investigated using calorimetry and were interpreted with crystal structures. The double **mutant**, in which Glu-86 and Ala-92 were replaced with Asp, clearly showed Ca2+ binding affinity, whereas neither point **mutant** showed Ca2+ affinity, indicating that both residues are essential. The further **mutation** of Ala-83 --> Lys did not affect the Ca2+ binding of the double **mutant** . The point **mutations** Ala-83 --> Lys and Glu-86 --> Asp did not affect the stability, whereas the **mutation** Ala-92 --> Asp was about 1.3 kcal/mol less stable. Structural analyses showed that both Asp-86 and Lys-83 were exposed to solvent. Side chains of Asp-86 and Asp-91 were rotated in opposite directions about chi1 angle, as if to reduce the electrostatic repulsion. The charged amino acids at the Ca2+ binding site did not significantly affect stability of the protein, possibly because of the local conformational change of the side chains.

L14 ANSWER 27 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1999:53902 SCISEARCH
THE GENUINE ARTICLE: 153TH
TITLE: Calmodulin binding to myosin light chain kinase begins at substoichiometric Ca2+ concentrations: A small-angle scattering study of binding and conformational transitions
AUTHOR: Krueger J K; Bishop N A; Blumenthal D K; Zhi G; Beckingham K; Stull J T; Trewhella J (Reprint)
CORPORATE SOURCE: LOS ALAMOS NATL LAB, CHEM SCI & TECHNOL DIV, MAIL STOP G758, LOS ALAMOS, NM 87545 (Reprint); LOS ALAMOS NATL LAB, CHEM SCI & TECHNOL DIV, LOS ALAMOS, NM 87545; RICE UNIV, DEPT BIOCHEM & CELL BIOL, HOUSTON, TX 77251; UNIV TEXAS, SW MED CTR, DEPT PHYSIOL, DALLAS, TX 75235; UNIV UTAH, DEPT PHARMACOL & TOXICOL, SALT LAKE CITY, UT 84112
COUNTRY OF AUTHOR: USA
SOURCE: BIOCHEMISTRY, (22 DEC 1998) Vol. 37, No. 51, pp. 17810-17817.

Searcher : Shears 571-272-2528

Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 53

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have used small-angle scattering to study the **calcium** dependence of the interactions between calmodulin (CaM) and skeletal muscle myosin light chain kinase (MLCK), as well as the conformations of the complexes that form. Scattering data were measured from equimolar mixtures of a functional MLCK and CaM or a **mutated** CaM (B12QCaM) incompetent to bind Ca2+ in its N-terminal domain, with increasing Ca2+ concentrations. To evaluate differences between CaM-enzyme versus CaM-peptide interactions, similar Ca2+ titration experiments were performed using synthetic peptides based on the CaM-binding sequence from MLCK (MLCK-I), Our data show there are different determinants for CaM binding the isolated peptide sequence compared to CaM binding to the same sequences within the enzyme. For example, binding of either CaM or B12QCaM to the MLCK-I peptide is observed even in the presence of EGTA, whereas binding of CaM to the enzyme requires Ca2+. The peptide studies also show that the conformational collapse of CaM requires both the N and C domains of CaM to be competent for Ca2+ binding as well as interactions with each end of MLCK-I, and it occurs at similar to 2 mol of Ca2+/mol of CaM. We show that CaM binding to the MLCK enzyme begins at substoichiometric concentrations of Ca2+ (less than or equal to 2 mol of Ca2+/mol of CaM), but that the final compact structure of CaM with the enzyme requires saturating Ca2+. in addition, MLCK enzyme does bind to 2Ca(2+). B12QCaM, although this complex is more extended than the complex with native CaM, Our results support the hypothesis that CaM regulation of MLCK involves an initial binding step at less than saturating Ca2+ concentrations and a subsequent activation step at higher Ca2+ concentrations.

L14 ANSWER 28 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 1998:418039 SCISEARCH
THE GENUINE ARTICLE: ZP874
TITLE: Equilibrium and kinetic folding of pigeon lysozyme
AUTHOR: Haezebrouck P; Noyelle K; VanDael H (Reprint)
CORPORATE SOURCE: KATHOLIEKE UNIV LEUVEN, INTERDISCIPLINARY RES CTR, CAMPUS KORTRIJK, B-8500 KORTRIJK, BELGIUM (Reprint); KATHOLIEKE UNIV LEUVEN, INTERDISCIPLINARY RES CTR, B-8500 KORTRIJK, BELGIUM
COUNTRY OF AUTHOR: BELGIUM
SOURCE: BIOCHEMISTRY, (12 MAY 1998) Vol. 37, No. 19, pp. 6772-6780.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036.
ISSN: 0006-2960.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 54

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In the present study, the search for a possible intermediate state in pigeon lysozyme is addressed by equilibrium and kinetic experiments

using static and stopped-flow fluorescence and circular dichroism spectroscopies. In equilibrium conditions at pH 7.5, pigeon lysozyme shows no populated intermediate state in temperature-and GdnHCl-induced unfolding experiments. In the unfolding process at low pH, however, a distinct intermediate state with molten globule characteristics is observed. CA(2+) binding to the protein is found to stabilize the native state. The early folding intermediate observed in kinetic experiments corresponds to the equilibrium intermediate in that an important amount of secondary structure has already been established. Full accomplishment of native tertiary contacts is achieved in a fast exponential process with a rate constant (0.23-135 s(-1)) that is strongly dependent on refolding conditions. Binding experiments with the fluorescent inhibitor MeU-diNAG support these conclusions. The folding rate is not influenced by Ca2+ binding. Analysis of the refolding and unfolding kinetics determined as a function of denaturant concentration leads to a Gibbs energy profile with a rate-determining transition state between the N- and I-states. Comparison with previous results on the folding of hen egg white lysozyme emphasizes the crucial role of Trp 62 in stabilizing non-native interactions. The replacement of this residue by Tyr in pigeon lysozyme contributes to the formation of native tertiary contacts.

L14 ANSWER 29 OF 41 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1998127758 EMBASE
TITLE: Structural evidence for the presence of a secondary **calcium** binding site in human α-**lactalbumin**.
AUTHOR: Chandra N.; Brew K.; Acharya K.R.
CORPORATE SOURCE: K.R. Acharya, Department of Biology/Biochemistry, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom. K.R.Acharya@bath.ac.uk
SOURCE: Biochemistry, (7 Apr 1998) Vol. 37, No. 14, pp. 4767-4772.
Refs: 44
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 19980611

AB The high-resolution X-ray crystal structure of human α-**lactalbumin** (at 1.8 Å) in the presence of an elevated level of **calcium** reveals a new secondary **calcium** binding site, 7.9 Å away from the primary **calcium** binding site known in all α- **lactalbumin** structures so far. The new **calcium** binding site is different from the zinc and sulfate binding sites [Ren, J., et al. (1993) J. Biol, Chemical 268, 19292-19298] but shares common features with the manganese binding site as described by Gerkin [Gerkin, T. A. (1984) Biochemistry 23, 4688-4697]. The proximity of the manganese and **calcium** binding region and the location of the functional site on one side of the charged surface of the α- **lactalbumin** molecule suggest that these binding sites might play a role in the formation of the lactose synthase complex.

L14 ANSWER 30 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1998:676311 SCISEARCH
THE GENUINE ARTICLE: 115KX
TITLE: Calorimetric study of **mutant** human lysozymes with partially introduced Ca2+ binding sites and its efficient refolding system from inclusion bodies
AUTHOR: Koshiba T; Tsumoto K; Masaki K; Kawano K; Nitta K (Reprint); Kumagai I
CORPORATE SOURCE: HOKKAIDO UNIV, GRAD SCH SCI, DIV BIOL SCI, KITA KU, SAPPORO, HOKKAIDO 060, JAPAN (Reprint); HOKKAIDO UNIV, GRAD SCH SCI, DIV BIOL SCI, KITA KU, SAPPORO, HOKKAIDO 060, JAPAN; TOHOKU UNIV, GRAD SCH ENGN, DEPT BIOCHEM & ENGN, AOBA KU, SENDAI, MIYAGI 98077, JAPAN
COUNTRY OF AUTHOR: JAPAN
SOURCE: PROTEIN ENGINEERING, (AUG 1998) Vol. 11, No. 8, pp. 683-690.
Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.
ISSN: 0269-2139.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During the process of evolution, ancestral lysozymes evolved into **calcium**-binding lysozymes by acquiring three critical aspartate residues at positions 86, 91 and 92. To investigate the process of the acquisition of **calcium**-binding ability, two of the aspartates were partially introduced into human lysozyme at positions 86, 91 and 92. These **mutants** (HLQ86D, HLA92D and HLQ86D/D91Q/A92D), having two critical aspartates in **calcium** -binding sites, were expressed in Escherichia coli as non-active inclusion bodies. For the preparation of lysozyme samples, a refolding system using thioredoxin was established. This system allowed for effective refolding of wild-type and **mutant** lysozymes, and 100% of activity was recovered within 4 days. The **calcium** ion dependence of the melting temperature (T-m) of wild-type and **mutant** lysozymes was investigated by differential scanning calorimetry at pH 4.5. The T-m values of wildtype, HLQ86D and HLA92D **mutants** were not dependent on **calcium** ion concentration. However, the T-m of HLQ86D/D910/A92D was 4 degrees higher in the presence of 50 mM CaCl2 than in its absence, and the **calcium**-binding constant of this **mutant** was estimated to be 2.25(+/-0.25)x10(2) M-1 at pH 4.5, Moreover, the **calcium**-binding ability of this **mutant** was confirmed by the result using Sephadex G-25 gel chromatography, These results indicate that it is indispensable to have at least two aspartates at positions 86 and 92 for acquisition of **calcium**-binding ability. The process of the acquisition of **calcium**-binding site during evolution of **calcium**-binding lysozyme is discussed.

L14 ANSWER 31 OF 41 MEDLINE on STN DUPLICATE 14
ACCESSION NUMBER: 97452583 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9305954
TITLE: Functional identification of **calcium** binding residues in bovine alpha-**lactalbumin**.
AUTHOR: Anderson P J; Brooks C L; Berliner L J

CORPORATE SOURCE: Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, USA.
SOURCE: Biochemistry, (1997 Sep 30) 36 (39) 11648-54. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023

AB The functional role of previously identified **calcium** binding residues in alpha-**lactalbumin** (alpha-LA) was investigated by site-directed **mutagenesis**. **Mutation** of **D82** to alanine did not effect the binding affinity for **calcium**, the protein structure, or its function in the lactose synthase assay, suggesting that this aspartate side chain is not essential for **calcium** binding or structural stabilization. In contrast, **mutation** of either **D87** or **D88** to alanine completely eliminated the strong **calcium** binding and altered alpha-LA as shown by several spectroscopically derived properties such as near- and far-UV CD and intrinsic fluorescence studies. These latter two **mutants** displayed significantly reduced abilities to stimulate lactose synthase activity (<3.5% of the maximal rate). Additionally, residues **K79** and **D84**, which chelate **calcium** by backbone carbonyls, were **mutated** to alanine. K79A lost approximately 50% of its tertiary structure and stability (as determined by CD) but retained full **calcium** binding activity, indicating that at least the lysine side chain does not influence the carbonyl-mediated **calcium** coordination. In contrast, D84A lost approximately 25% of its tertiary structure and stability which was accompanied by a modest reduction in **calcium** affinity. Both **mutants** were able to stimulate normal lactose synthase activity. The triple **mutant**, D82A/**D87A**/D88A alpha-LA, lost its ability to bind **calcium**, similar to **D87A** and D88A. These studies clearly demonstrate the importance and variation of side chain interactions, which might be the seminal event in the establishment of the correct **calcium** binding loop conformation, possibly to stabilization and final folding of the overall protein structure.

L14 ANSWER 32 OF 41 MEDLINE on STN DUPLICATE 15
ACCESSION NUMBER: 97141754 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8988012
TITLE: Thermodynamic characterization of the partially unfolded state of Ca(2+)-loaded bovine alpha-**lactalbumin**: evidence that partial unfolding can precede Ca2+ release.
AUTHOR: Vanderheeren G; Hanssens I; Meijberg W; Van Aerschot A
CORPORATE SOURCE: Interdisciplinary Research Center, Katholieke Universiteit Leuven, Kortrijk, Belgium.
SOURCE: Biochemistry, (1996 Dec 24) 35 (51) 16753-9, Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701

Searcher : Shears 571-272-2528

ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

AB The thermal denaturation of bovine alpha-**lactalbumin** (BLA) was studied at pH 7.5 and at various Ca2+ concentrations using near-UV circular dichroism and differential scanning calorimetry. The Ca2+ dependence of the denaturation equilibria proves that, in the transition region, partially unfolded alpha-**lactalbumin** consists of a mixture of Ca(2+)-loaded and Ca(2+)-free protein. The thermodynamic parameters of the unfolding of these two species were determined at 68 degrees C and were then compared with one other, with the thermodynamic parameters deduced from calorimetric titration of alpha-**lactalbumin** with Ca2+, and with those derived from Ca2+ titration of a **mutant** human lysozyme having an engineered Ca(2+)-binding site. This comparison indicated that (a) the unfolding curves for Ca(2+)-BLA deduced from the near-UV ellipticity change are more able to distinguish between unfolding with and without Ca2+ release than those deduced from differential scanning calorimetry, (b) the Ca(2+)-loaded denaturated state of BLA is more folded than the Ca(2+)-free protein at 68 degrees C, and (c) a heat-induced unfolding process, consisting of an initial Ca2+ release, followed by a conformational relaxation, is unlikely to occur at the experimental pH and in the millimolar region of Ca2+ concentrations, due to the large free energy requirement of the initial step. A more probable mechanism would be unfolding via a Ca(2+)-loaded intermediately unfolded state, with subsequent Ca2+ release.

L14 ANSWER 33 OF 41 MEDLINE on STN
ACCESSION NUMBER: 95256211 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7737986
TITLE: A Ca(2+)-binding chimera of human lysozyme and bovine alpha-**lactalbumin** that can form a molten globule.
AUTHOR: Pardon E; Haezebrouck P; De Baetselier A; Hooke S D; Fancourt K T; Desmet J; Dobson C M; Van Dael H; Joniau M
CORPORATE SOURCE: Interdisciplinary Research Center, K. U. Leuven, Kortrijk, Belgium.
SOURCE: Journal of biological chemistry, (1995 May 5) 270 (18) 10514-24.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950615
Last Updated on STN: 19980206
Entered Medline: 19950605

AB In contrast to lysozymes, which undergo **two-state thermal** denaturation, the Ca(2+)-free form of the homologous **alpha-lactalbumins** forms an intermediate "molten globule" state. To understand this difference, we have produced a chimera of human lysozyme and bovine alpha-**lactalbumin**. In the synthetic gene of the former the sequence coding for amino acid residues 76-102 was replaced by that for bovine alpha-**lactalbumin** 72-97, which represents the Ca(2+)-binding loop and the central helix C. The chimeric protein, LYLA1, expressed in Saccharomyces cerevisiae was homogeneous on electrophoresis and mass spectrometry. Its Ca2+

binding constant was 2.50 (+/- 0.04) x 10(8) M-1, and its muramidase activity 10% of that of human lysozyme. One-dimensional NMR spectroscopy indicated the presence of a compact, well structured protein. From two-dimensional NMR spectra, main chain resonances for 118 of a total of 129 residues could be readily assigned. Nuclear Overhauser effect analysis and hydrogen-deuterium exchange measurements indicated the presence and persistence of all expected secondary structure elements. Thermal denaturation, measured by circular dichroism, showed a single transition temperature for the Ca2+ form at 90 degrees C, whereas unfolding of the apo form occurred at 73 degrees C in the near-UV and 81 degrees C in the far-UV range. These observations illustrate that by transplanting the central part of bovine alpha-**lactalbumin**, we have introduced into human lysozyme two important properties of alpha-**lactalbumins**, i.e. stabilization through Ca2+ binding and molten globule behavior.

L14 ANSWER 34 OF 41 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 94294417 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8022817
TITLE: Creation and phenotypic analysis of alpha-**lactalbumin**-deficient mice.
AUTHOR: Stinnakre M G; Vilotte J L; Soulier S; Mercier J C
CORPORATE SOURCE: Laboratoire de Genetique Biochimique et de Cytogenetique, Institut National de la Recherche Agronomique, Jouy-en-Josas, France.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1994 Jul 5) 91 (14) 6544-8. Journal code: 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940815
Last Updated on STN: 19940815
Entered Medline: 19940801

AB alpha-**Lactalbumin** is an abundant milk-specific **calcium** metalloprotein which has an evolutionary relationship to lysozyme. It modifies the substrate specificity of a Golgi galactosyltransferase by forming the lactose synthetase binary complex. Lactose, together with other sugars and diffusible ions, is responsible for the osmotic pressure of milk. To assess the involvement of alpha-**lactalbumin** in lactogenesis, alpha-**lactalbumin**-deficient mice were created by disrupting the gene by homologous recombination in embryonic stem cells. Homozygous **mutant** mice are viable and fertile but females cannot **feed** their offspring. They produce a highly viscous milk that pups appear to be unable to remove from the mammary gland. This milk is rich in fat and protein and is devoid of alpha-**lactalbumin** and lactose. The phenotype of heterozygous mice was found to be intermediate, with a 40% decrease in alpha-**lactalbumin** but only a 10-20% decrease in the lactose content of their milk compared with wild-type animals. These results emphasize the key function of alpha-**lactalbumin** in lactogenesis and open new opportunities to manipulate milk composition.

L14 ANSWER 35 OF 41 MEDLINE on STN DUPLICATE 17
ACCESSION NUMBER: 94052076 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8234235

TITLE: Stability effects associated with the introduction of a partial and a complete Ca(2+)-binding site into human lysozyme.
AUTHOR: Haezebrouck P; De Baetselier A; Joniau M; Van Dael H; Rosenberg S; Hanssens I
CORPORATE SOURCE: Interdisciplinary Research Center, Katholieke Universiteit Leuven, Kortrijk, Belgium.
SOURCE: Protein engineering, (1993 Aug) 6 (6) 643-9.
Journal code: 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19940117
Entered Medline: 19931208

AB Two **mutants** of human lysozyme were synthesized. **Mutant** A92D, in which Ala92 was substituted by Asp, contains a partial Ca(2+)-binding site and **mutant** M4, in which Ala83, Gln86, Asn88 and Ala92 were replaced by Lys, Asp, Asp and Asp respectively, contains the complete Ca(2+)-binding site of bovine alpha-**lactalbumin**. The Ca(2+)-binding constants of wild type human lysozyme and of **mutants** A92D and M4, measured at 25 degrees C and pH 7.5, were 2(+/- 1) x 10(2) M-1, 8(+/- 2) x 10(3) M-1 and 9(+/- 0.5) x 10(6) M-1 respectively. Information gathered from microcalorimetric and CD spectroscopic measurements indicates that the conformational changes of the M4 **mutant** lysozyme, induced by Ca2+ binding, are smaller than those observed for bovine alpha-**lactalbumin** and for the Ca(2+)-binding equine lysozyme. At pH 4.5, the thermostability of both the apo and Ca2+ forms of the A92D human was decreased in comparison with that of native human lysozyme. In particular, within the apo form of this **mutant** an alpha-helix-containing sequence was destabilized. In contrast, at the same pH the thermostability of the apo and Ca2+ forms of the M4 **mutant** lysozyme was increased. The epsilon-ammonium group of the Lys83 side chain is assumed to be responsible for the stabilization of the apo form of this **mutant**.

L14 ANSWER 36 OF 41 MEDLINE on STN DUPLICATE 18
ACCESSION NUMBER: 93077511 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1447179
TITLE: Thermodynamic changes in the binding of Ca2+ to a **mutant** human lysozyme (D86/92). Enthalpy-entropy compensation observed upon Ca2+ binding to proteins.
AUTHOR: Kuroki R; Nitta K; Yutani K
CORPORATE SOURCE: Protein Engineering Research Institute, Osaka, Japan.
SOURCE: Journal of biological chemistry, (1992 Dec 5) 267 (34) 24297-301.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19930129

Searcher : Shears 571-272-2528

Entered Medline: 19921230

AB The thermodynamic change in the binding of Ca2+ to a **mutant** human lysozyme having an engineered Ca2+ binding site (Kuroki, R., Taniyama, Y., Seko, C., Nakamura, H., Kikuchi, M., and Ikehara, M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6903-6907) was analyzed by calorimetry and interpreted in terms of structural information obtained from x-ray crystallography. It was found that the enthalpic contribution for the Ca2+ binding reaction was small, driven primarily by entropy release (10 kcal/mol). This release of entropy was also observed in some organic chelators. Moreover, through the information of the tertiary structures of the apo- and holomutant lysozyme, it was confirmed that the entropy release (10 kcal/mol) upon the binding of Ca2+ arises primarily from the release of bound water molecules hydrating the free Ca2+. Previous studies of Ca2+ binding to proteins have involved significant changes in protein conformation. They can now be reevaluated to determine the contribution of conformational changes to Ca2+ binding. After removing the thermodynamic contribution of Ca2+ binding itself, it is found that upon the binding of Ca2+ the enthalpy change is negative but is almost compensated by the negative entropy change. The negative change in both enthalpy and entropy is characteristic of values seen in the thermodynamic change upon the folding of proteins.

L14 ANSWER 37 OF 41 MEDLINE on STN DUPLICATE 19
ACCESSION NUMBER: 92369115 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1504092
TITLE: Hydrophobic interaction of lysozyme and alpha-**lactalbumin** from equine milk whey.
AUTHOR: Haezebrouck P; Noppe W; Van Dael H; Hanssens I
CORPORATE SOURCE: Interdisciplinary Research Center, K.U.L. Campus Kortrijk, Belgium.
SOURCE: Biochimica et biophysica acta, (1992 Aug 21) 1122 (3) 305-10.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19921009
Last Updated on STN: 19921009
Entered Medline: 19920922

AB From fluorescence measurements on mixtures of bis-ANS and equine lysozyme and from Ca(2+)-dependent hydrophobic interaction chromatography of equine lysozyme, it is demonstrated that Ca2+ binding induces a conformational change upon which hydrophobic regions in the protein become less accessible. Bis-ANS fluorescence titrations in the absence of Ca2+ and in 2 mM Ca2+ are also performed with equine alpha-**lactalbumin** variants B and C. These variants differ by an amino-acid exchange Asp----Ile at residue 95. The fluorescence titration curves indicate that the accessibility of the probe to the Ca2+ conformers is clearly influenced by the **mutation**. The Ca(2+)-dependent exclusion of a hydrophobic domain is used in a new and simplified method for preparing lysozyme and alpha-**lactalbumins** simultaneously from equine milk whey.

L14 ANSWER 38 OF 41 MEDLINE on STN DUPLICATE 20
ACCESSION NUMBER: 92041917 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1939116

TITLE: Crystal structures of the apo- and holomutant human lysozymes with an introduced Ca2+ binding site.
AUTHOR: Inaka K; Kuroki R; Kikuchi M; Matsushima M
CORPORATE SOURCE: Protein Engineering Research Institute, Osaka, Japan.
SOURCE: Journal of biological chemistry, (1991 Nov 5) 266 (31) 20666-71.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199112
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19911213

AB The three-dimensional structures of apo- and holomutant human lysozymes (D86/92 lysozyme), in which a **calcium** binding site was designed and created for enhancing molecular stability by replacing both Gln86 and Ala92 with aspartic acids, were refined at 1.8-A resolution by x-ray crystallography. The overall structures and crystallographic thermal factors of all three proteins, the apo-, holo-D86/92, and the wild-type human lysozymes, were essentially identical; these results showed that the introduction of the **calcium** binding site did not affect either the overall structure or molecular rigidity of the proteins. However, structure analyses of the apo-D86/92 lysozyme revealed that the **mutations** affected the side chain conformation of residue 86 and hydrogen networks between the protein and the internal solvent molecules. In the structure of the holo-D86/92 lysozyme, seven oxygen ligands formed a slightly distorted pentagonal bipyramid around the **calcium** ion, indicating that the coordination around the **calcium** ion was quite similar to that in baboon alpha-**lactalbumin**. The pentagonal bipyramid coordination could be one of the most widely found and appropriate **calcium** binding schemes in proteins.

L14 ANSWER 39 OF 41 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 91:79024 SCISEARCH
THE GENUINE ARTICLE: EV677
TITLE: CDNA AND AMINO-ACID-SEQUENCES OF RAINBOW-TROUT (ONCORHYNCHUS-MYKISS) LYSOZYMES AND THEIR IMPLICATIONS FOR THE EVOLUTION OF LYSOZYME AND **LACTALBUMIN**
AUTHOR: DAUTIGNY A; PRAGER E M; PHAMDINH D; JOLLES J; PAKDEL F; GRINDE B; JOLLES P (Reprint)
CORPORATE SOURCE: UNIV PARIS 05, PROT LAB, 45 RUE ST PERES, F-75270 PARIS 06, FRANCE; UNIV RENNES 1, MOLEC BIOL LAB, F-35000 RENNES, FRANCE; UNIV CALIF BERKELEY, DIV BIOCHEM & MOLEC BIOL, BERKELEY, CA, 94720; NATL INST PUBL HLTH, N-0462 OSLO 4, NORWAY
COUNTRY OF AUTHOR: FRANCE; USA; NORWAY
SOURCE: JOURNAL OF MOLECULAR EVOLUTION, (1991) Vol. 32, No. 2, pp. 187-198.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 29
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The complete 129-amino-acid sequences of two rainbow trout

lysozymes (I and II) isolated from kidney were established using protein chemistry microtechniques. The two sequences differ only at position 86, I having aspartic acid and II having alanine. A cDNA clone coding for rainbow trout lysozyme was isolated from a cDNA library made from liver mRNA. Sequencing of the cloned cDNA insert, which was 1 kb in length, revealed a 432-bp open reading frame encoding an amino-terminal peptide of 15 amino acids and a mature enzyme of 129 amino acids identical in sequence to II. Forms I and II from kidney and liver were also analyzed using enzymatic amplification via PCR and direct sequencing; both organs contain mRNA encoding the two lysozymes. Evolutionary trees relating DNA sequences coding for lysozymes c and alpha-**lactalbumins** provide evidence that the gene duplication giving rise to conventional vertebrate lysozymes c and to **lactalbumin** preceded the divergence of fishes and tetrapods about 400 Myr ago. Evolutionary analysis also suggests that amino acid replacements may have accumulated more slowly on the lineage leading to fish lysozyme than on those leading to mammal and bird lysozymes.

L14 ANSWER 40 OF 41 MEDLINE on STN
ACCESSION NUMBER: 89276372 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2731545
TITLE: The evolution of lysozyme and alpha-**lactalbumin**
.
AUTHOR: Nitta K; Sugai S
CORPORATE SOURCE: Department of Polymer Science, Faculty of Science, Hokkaido University, Japan.
SOURCE: European journal of biochemistry / FEBS, (1989 Jun 1) 182 (1) 111-8.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198907
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19890718

AB From the analysis of phylogenetic trees constructed from the amino acid sequences and metal-binding properties of various lysozymes c and alpha-**lactalbumins**, it was found that before the divergence of the lineages of birds and mammals, **calcium**-binding lysozyme diverged from non-**calcium**-binding lysozyme. alpha-**Lactalbumin** evolved from the **calcium**-binding lysozyme along the mammalian lineage after the divergence of birds and mammals. Rapid evolution took place, not in the process of acquisition of the activity of alpha-**lactalbumin**, but after the loss of lysozyme activity, due to the change in the distribution of selective pressure on each amino acid site. A general process for the change in function of a protein during evolution is suggested to be as follows: after duplication of the gene, one of their protein products acquires a new function, besides that already present; the old function is eventually lost.

L14 ANSWER 41 OF 41 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1985:294143 BIOSIS
DOCUMENT NUMBER: PREV198579074139; BA79:74139
TITLE: THE PRODUCTION OF COLLAGENASE BY ADHERENT MONONUCLEAR

CELLS CULTURED FROM HUMAN PERIPHERAL BLOOD.

AUTHOR(S): LOUIE J S [Reprint author]; WEISS J; RYHANEN L; NIES K M; RANTALA-RYHANEN S; UITTO J

CORPORATE SOURCE: DIVISION RHEUMATOLOGY, DEPARTMENT MEDICINE, HARBOR-UCLA MEDICAL CENTER, UCLA SCHOOL MEDICINE, 1000 WEST CARSON STREET, TORRANCE, CALIF 90509, USA

SOURCE: Arthritis and Rheumatism, (1984) Vol. 27, No. 12, pp. 1397-1404.
CODEN: ARHEAW. ISSN: 0004-3591.

DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Mononuclear cells were isolated from human peripheral blood by Ficoll-Hypaque centrifugation, and the cells adherent to plastic substrata were cultured in serum-free media supplemented with **lactalbumin** hydrolysate. These cell cultures, which consisted predominantly of monocyte-macrophages as judged by nonspecific esterase staining, accumulated collagenase in the medium. This collagenase resembled other vertebrate collagenases in that it cleaved native triple-helical type I collagen at a locus 3/4-length away from the amino-terminal end of the molecule. The collagenase activity was inhibited by NA2EDTA, dithiothreitol and fetal calf serum, while the addition of Ca2+ or N-ethylmaleimide enhanced the enzyme activity. The accumulation of collagenase in the culture media was markedly enhanced by the incubation of cells with concanavalin A or phorbol myristic acetate. In the presence of cycloheximide, the levels of collagenase activity were markedly reduced, suggesting that active protein synthesis was required to express the enzyme activity. In additional experiments, monocytes were further purified by counterflow centrifugation-elutriation. The collagenase production was markedly increased in cultures enriched in monocyte-macrophages and devoid of **polymorphonuclear** leukocytes. The accumulation of collagenase in monocyte cultures incubated for 48 h in the presence of concanavalin A or phorbol myristic acetate was of the same order of magnitude as in parallel cultures containing the same number of **polymorphonuclear** leukocytes purified by Ficoll-Hypaque centrifugation and Plasmagel sedimentation. The demonstration of collagenase activity in the monocyte cultures appears to reflect the increased diversity of monocyte functions which may play an important role in the tissue damage in chronic inflammatory diseases such as rheumatoid arthritis.

FILE 'CAPLUS' ENTERED AT 15:20:41 ON 08 APR 2005
L15 2 S L6 AND ((C18 OR C 18)(W)1)(S)FATTY ACID
L16 0 S L15 NOT L9

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, CANCERLIT' ENTERED AT 15:21:51 ON 08 APR 2005)
L17 4 S L10 AND ((C18 OR C 18)(W)1)(S)FATTY ACID
L18 2 S L17 NOT L13
L19 2 DUP REM L18 (0 DUPLICATES REMOVED)

L19 ANSWER 1 OF 2 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2004184504 EMBASE
TITLE: Conformational analysis of HAMLET, the **folding variant** of human α- **lactalbumin** associated with apoptosis.
AUTHOR: Casbarra A.; Birolo L.; Infusini G.; Dal Piaz F.;

Svensson M.; Pucci P.; Svanborg C.; Marino G.
CORPORATE SOURCE: G. Marino, Dipto. di Chim. Organ. e Biochimica, Universita di Napoli Federico II, Via Cinthia, I-80126 Napoli, Italy. gmarino@unina.it
SOURCE: Protein Science, (2004) Vol. 13, No. 5, pp. 1322-1330.
Refs: 29
ISSN: 0961-8368 CODEN: PRCIEI
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040528
Last Updated on STN: 20040528

AB A combination of hydrogen/deuterium (H/D) exchange and limited proteolysis experiments coupled to mass spectrometry analysis was used to depict the conformation in solution of HAMLET, the folding variant of human α- **lactalbumin**, complexed to oleic acid, that induces apoptosis in tumor and immature cells. Although near- and far-UV CD and fluorescence spectroscopy were not able to discriminate between HAMLET and apo-α- **lactalbumin**, H/D exchange experiments clearly showed that they correspond to two distinct conformational states, with HAMLET incorporating a greater number of deuterium atoms than the apo and holo forms. Complementary proteolysis experiments revealed that HAMLET and apo are both accessible to proteases in the β-domain but showed substantial differences in accessibility to proteases at specific sites. The overall results indicated that the conformational changes associated with the release of Ca(2+) are not sufficient to induce the HAMLET conformation. Metal depletion might represent the first event to produce a partial unfolding in the β-domain of α- **lactalbumin**, but some more unfolding is needed to generate the active conformation HAMLET, very likely allowing the protein to bind the **C18:1 fatty acid** moiety.
On the basis of these data, a putative binding site of the oleic acid, which stabilizes the HAMLET conformation, is proposed.

L19 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2000:240386 BIOSIS
DOCUMENT NUMBER: PREV200000240386
TITLE: Conversion of alpha-**lactalbumin** to a protein inducing apoptosis.
AUTHOR(S): Svensson, M.; Hakansson, A.; Mossberg, A.-K.; Linse, S.; Svanborg, C. [Reprint author]
CORPORATE SOURCE: Department of Microbiology, Immunology and Glycobiology (MIG), Institute of Laboratory Medicine, Lund University, Solvegatan 23, S-223 62, Lund, Sweden
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (April 11, 2000) Vol. 97, No. 8, pp. 4221-4226. print.
CODEN: PNASA6. ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Jun 2000
Last Updated on STN: 5 Jan 2002

AB In this study alpha-**lactalbumin** was converted from the regular, native state to a folding variant with altered biological function. The folding variant was shown to induce apoptosis in tumor

cells and immature cells, but healthy cells were resistant to this effect. Conversion to HAMLET (human alpha-**lactalbumin** made lethal to tumor cells) required partial unfolding of the protein and a specific **fatty acid, C18:1,** as a necessary cofactor. Conversion was achieved with alpha-**lactalbumin** derived from human milk whey and with recombinant protein expressed in Escherichia coli. We thus have identified the folding change and the fatty acid as two key elements that define HAMLET, the apoptosis-inducing functional state of alpha-**lactalbumin**. Although the environment in the mammary gland favors the native conformation of alpha-**lactalbumin** that serves as a specifier in the lactose synthase complex, the conditions under which HAMLET was formed resemble those in the stomach of the nursing child. Low pH is known to release Ca2+ from the high-affinity Ca2+-binding site and to activate lipases that hydrolyze free fatty acids from milk triglycerides. We propose that this single amino acid polypeptide chain may perform vastly different biological functions depending on its folding state and the in vivo environment. It may be speculated that molecules like HAMLET can aid in lowering the incidence of cancer in breast-fed children by purging of tumor cells from the gut of the neonate.

FILE 'MEDLINE' ENTERED AT 15:23:09 ON 08 APR 2005
L20 1804 SEA FILE=MEDLINE ABB=ON PLU=ON LACTALBUMIN/CT
L21 12 SEA FILE=MEDLINE ABB=ON PLU=ON L20 AND ("FATTY ACIDS")/CT

L20 1804 SEA FILE=MEDLINE ABB=ON PLU=ON LACTALBUMIN/CT
L22 162 SEA FILE=MEDLINE ABB=ON PLU=ON L20 AND CALCIUM/CT
L23 4 SEA FILE=MEDLINE ABB=ON PLU=ON L22 AND (MUTATION OR MUTAGENESIS OR "POLYMORPHISM, GENETIC")/CT

L24 16 L21 OR L23

L24 ANSWER 1 OF 16 MEDLINE on STN
ACCESSION NUMBER: 2003561339 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14627739
TITLE: Alpha-lactalbumin unfolding is not sufficient to cause apoptosis, but is required for the conversion to HAMLET (human alpha-lactalbumin made lethal to tumor cells).
AUTHOR: Svensson Malin; Fast Jonas; Mossberg Ann-Kristin; Duringer Caroline; Gustafsson Lotta; Hallgren Oskar; Brooks Charles L; Berliner Lawrence; Linse Sara; Svanborg Catharina
CORPORATE SOURCE: Department of Microbiology, Immunology and Glycobiology (MIG), Institute of Laboratory Medicine, Lund University, Lund, Sweden.
SOURCE: Protein science : a publication of the Protein Society, (2003 Dec) 12 (12) 2794-804.
Journal code: 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200407
ENTRY DATE: Entered STN: 20031216
Last Updated on STN: 20040715
Entered Medline: 20040714
ED Entered STN: 20031216

Last Updated on STN: 20040715
Entered Medline: 20040714

AB HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a complex of human alpha-lactalbumin and oleic acid (C18:1:9 cis) that kills tumor cells by an apoptosis-like mechanism. Previous studies have shown that a conformational change is required to form HAMLET from alpha-lactalbumin, and that a partially unfolded conformation is maintained in the HAMLET complex. This study examined if unfolding of alpha-lactalbumin is sufficient to induce cell death. We used the bovine alpha-lactalbumin Ca(2+) site mutant D87A, which is unable to bind Ca(2+), and thus remains partially unfolded regardless of solvent conditions. The D87A mutant protein was found to be inactive in the apoptosis assay, but could readily be converted to a HAMLET-like complex in the presence of oleic acid. BAMLET (bovine alpha-lactalbumin made lethal to tumor cells) and D87A-BAMLET complexes were both able to kill tumor cells. This activity was independent of the Ca(2+)site, as HAMLET maintained a high affinity for Ca(2+) but D87A-BAMLET was active with no Ca(2+) bound. We conclude that partial unfolding of alpha-lactalbumin is necessary but not sufficient to trigger cell death, and that the activity of HAMLET is defined both by the protein and the lipid cofactor. Furthermore, a functional Ca(2+)-binding site is not required for conversion of alpha-lactalbumin to the active complex or to cause cell death. This suggests that the lipid cofactor stabilizes the altered fold without interfering with the Ca(2+)site.

L24 ANSWER 2 OF 16 MEDLINE on STN
ACCESSION NUMBER: 2000189636 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10727102
TITLE: Peptide analogs from E-cadherin with different calcium-binding affinities.
AUTHOR: Yang W; Tsai T; Kats M; Yang J J
CORPORATE SOURCE: Department of Biology, Georgia State University, Atlanta, USA.
SOURCE: journal of peptide research : official journal of the American Peptide Society, (2000 Mar) 55 (3) 203-15. Journal code: 9707067. ISSN: 1397-002X.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY DATE: Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000428

ED Entered STN: 20000512
Last Updated on STN: 20000512
Entered Medline: 20000428

AB Cadherins are a family of calcium-dependent cell-surface proteins that are fundamental in controlling the development and maintenance of tissues. Motif B of E-cadherin seems to be a crucial calcium-binding site as single point mutations (D134A and D134K) completely inactivate its adhesion activity. We analyzed peptide models corresponding to motif B (amino acids 128-144) as well as selected mutations of this motif. Our NMR studies showed that this motif B sequence is actually an active calcium-binding region, even in the absence of the rest of the cadherin molecule. We found that the binding affinity of this motif is very sensitive to mutations. For example, our peptide P128-144 with the native calcium-binding sequence has an affinity of

Kd 0.4 mM, whereas the mutants P128-144/ D134A and P128-144/D134K containing the replacement of Asp134 by Ala and Lys, have Kd values of only 1.5 and 11 mM, respectively. Removing Asp at position 134, which correlates with the loss of adhesion activity, decreases calcium-binding affinity 20-fold. Ala132, along with residues Asp134, Asp136 and Asn143, is involved in calcium binding in solution. We also demonstrated that the calcium-binding affinity can be increased 3-fold when an additional Asp is introduced at position 132. In 50% organic solvent, this binding affinity of peptide P128-144/A132D (17-mer) from E-cadherin is similar to that of peptide P72-100/C73-77-91A (29-mer) from alpha-lactalbumin.

L24 ANSWER 3 OF 16 MEDLINE on STN
ACCESSION NUMBER: 2000138361 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10672181
TITLE: A folding variant of alpha-lactalbumin with bactericidal activity against Streptococcus pneumoniae.
COMMENT: Erratum in: Mol Microbiol 2000 Apr;36(1):247
AUTHOR: Hakansson A; Svensson M; Mossberg A K; Sabharwal H; Linse S; Lazou I; Lonnerdal B; Svanborg C
CORPORATE SOURCE: Department of Microbiology, Immunology and Glycobiology, Institute of Laboratory Medicine, Lund University, Solvegatan 23, SE-223 62 Lund, Sweden.
SOURCE: Molecular microbiology, (2000 Feb) 35 (3) 589-600. Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000407
Last Updated on STN: 20000613
Entered Medline: 20000328
ED Entered STN: 20000407
Last Updated on STN: 20000613
Entered Medline: 20000328
AB This study describes an alpha-lactalbumin folding variant from human milk with bactericidal activity against antibiotic-resistant and -susceptible strains of Streptococcus pneumoniae. The active complex precipitated with the casein fraction at pH 4.6 and was purified from casein by a combination of anion exchange and gel chromatography. Unlike other casein components, the active complex was retained on the ion-exchange matrix and eluted only with high salt. The eluted fraction showed N-terminal and mass spectrometric identity with human milk alpha-lactalbumin, but native alpha-lactalbumin had no bactericidal effect. Spectroscopic analysis demonstrated that the active form of the molecule was in a different folding state, with secondary structure identical to alpha-lactalbumin from human milk whey, but fluctuating tertiary structure. Native alpha-lactalbumin could be converted to the active bactericidal form by ion-exchange chromatography in the presence of a cofactor from human milk casein, characterized as a C18:1 fatty acid. Analysis of the antibacterial spectrum showed selectivity for streptococci; Gram-negative and other Gram-positive bacteria were resistant. The folding variant of alpha-lactalbumin is a new example of naturally occurring molecules with antimicrobial activity.

L24 ANSWER 4 OF 16 MEDLINE on STN
ACCESSION NUMBER: 1998049545 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9388223
TITLE: Interactions of alpha-lactalbumin with fatty acids and spin label analogs.
AUTHOR: Cawthern K M; Narayan M; Chaudhuri D; Permyakov E A; Berliner L J
CORPORATE SOURCE: Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, USA.
SOURCE: Journal of biological chemistry, (1997 Dec 5) 272 (49) 30812-6.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY DATE: Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980108

ED Entered STN: 19980122
Last Updated on STN: 19980122
Entered Medline: 19980108

AB Bovine alpha-lactalbumin (alpha-LA) has been shown by intrinsic protein fluorescence and electron spin resonance methods to interact with the spin-labeled fatty acid analog, 5-doxylstearic acid, as well as stearic acid. An intrinsic fluorescence titration of various alpha-LA forms with 5-doxylstearic acid causes first an increase and then a decrease in emission intensity with concomitant shifts in tryptophan emission wavelength. In some cases, up to three steps in the fluorescence titration curves were visible, which were fit to apparent binding steps from 10(-6) to 10(-4) M. The binding parameters of 5-doxylstearic acid for apo- and Ca2+-alpha-LA were an order of magnitude different from one another; the stronger one, apo-alpha-lactalbumin, exhibited a Kd of 35 microM. Electron spin resonance titrations of 5-doxylstearic acid-loaded apo-alpha-LA with stearate (micelles) seem to suggest separate binding loci if alpha-LA indeed binds stearate at these concentrations. The titration of alpha-LA by stearic acid results in a fluorescence emission red shift and an apparent stepped increase in fluorescence intensity. Lipid-protein association occurred at concentrations at which stearic acid micelles and aggregates begin to form in the absence of protein. Nonetheless, the relatively strong association between stearic acid and apo-alpha-LA was also confirmed by means of the fluorescent indicator acrylodated fatty acid binding protein, in which addition of alpha-LA to the stearate-loaded indicator protein reverses the decrease in fluorescence of the acrylodan chromophore conjugated to the protein.

L24 ANSWER 5 OF 16 MEDLINE on STN
ACCESSION NUMBER: 97141754 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8988012
TITLE: Thermodynamic characterization of the partially unfolded state of Ca(2+)-loaded bovine alpha-lactalbumin: evidence that partial unfolding can precede Ca2+ release.
AUTHOR: Vanderheeren G; Hanssens I; Meijberg W; Van Aerschot A
CORPORATE SOURCE: Interdisciplinary Research Center, Katholieke Universiteit Leuven, Kortrijk, Belgium.
SOURCE: Biochemistry, (1996 Dec 24) 35 (51) 16753-9.
Journal code: 0370623. ISSN: 0006-2960.

Searcher : Shears 571-272-2528

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

ED Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

AB The thermal denaturation of bovine alpha-lactalbumin (BLA) was studied at pH 7.5 and at various Ca2+ concentrations using near-UV circular dichroism and differential scanning calorimetry. The Ca2+ dependence of the denaturation equilibria proves that, in the transition region, partially unfolded alpha-lactalbumin consists of a mixture of Ca(2+)-loaded and Ca(2+)-free protein. The thermodynamic parameters of the unfolding of these two species were determined at 68 degrees C and were then compared with one other, with the thermodynamic parameters deduced from calorimetric titration of alpha-lactalbumin with Ca2+, and with those derived from Ca2+ titration of a mutant human lysozyme having an engineered Ca(2+)-binding site. This comparison indicated that (a) the unfolding curves for Ca(2+)-BLA deduced from the near-UV ellipticity change are more able to distinguish between unfolding with and without Ca2+ release than those deduced from differential scanning calorimetry, (b) the Ca(2+)-loaded denaturated state of BLA is more folded than the Ca(2+)-free protein at 68 degrees C, and (c) a heat-induced unfolding process, consisting of an initial Ca2+ release, followed by a conformational relaxation, is unlikely to occur at the experimental pH and in the millimolar region of Ca2+ concentrations, due to the large free energy requirement of the initial step. A more probable mechanism would be unfolding via a Ca(2+)-loaded intermediately unfolded state, with subsequent Ca2+ release.

L24 ANSWER 6 OF 16 MEDLINE on STN
ACCESSION NUMBER: 93077511 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1447179
TITLE: Thermodynamic changes in the binding of Ca2+ to a mutant human lysozyme (D86/92). Enthalpy-entropy compensation observed upon Ca2+ binding to proteins.
AUTHOR: Kuroki R; Nitta K; Yutani K
CORPORATE SOURCE: Protein Engineering Research Institute, Osaka, Japan.
SOURCE: Journal of biological chemistry, (1992 Dec 5) 267 (34) 24297-301.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19930129
Entered Medline: 19921230

ED Entered STN: 19930129
Last Updated on STN: 19930129
Entered Medline: 19921230

AB The thermodynamic change in the binding of Ca2+ to a mutant human lysozyme having an engineered Ca2+ binding site (Kuroki, R., Taniyama,

Y., Seko, C., Nakamura, H., Kikuchi, M., and Ikehara, M. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6903-6907) was analyzed by calorimetry and interpreted in terms of structural information obtained from x-ray crystallography. It was found that the enthalpic contribution for the Ca2+ binding reaction was small, driven primarily by entropy release (10 kcal/mol). This release of entropy was also observed in some organic chelators. Moreover, through the information of the tertiary structures of the apo- and holomutant lysozyme, it was confirmed that the entropy release (10 kcal/mol) upon the binding of Ca2+ arises primarily from the release of bound water molecules hydrating the free Ca2+. Previous studies of Ca2+ binding to proteins have involved significant changes in protein conformation. They can now be reevaluated to determine the contribution of conformational changes to Ca2+ binding. After removing the thermodynamic contribution of Ca2+ binding itself, it is found that upon the binding of Ca2+ the enthalpy change is negative but is almost compensated by the negative entropy change. The negative change in both enthalpy and entropy is characteristic of values seen in the thermodynamic change upon the folding of proteins.

L24 ANSWER 7 OF 16 MEDLINE on STN
ACCESSION NUMBER: 92126036 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1772422
TITLE: Ca2+ and pH dependence of hydrophobicity of alpha-lactalbumin: affinity partitioning of proteins in aqueous two-phase systems containing poly(ethylene glycol) esters of fatty acids.
AUTHOR: Shanbhag V P; Johansson G; Ortin A
CORPORATE SOURCE: Department of Biochemistry, University of Umea, Sweden.
SOURCE: Biochemistry international, (1991 Jun) 24 (3) 439-50. Journal code: 8100311. ISSN: 0158-5231.
PUB. COUNTRY: Australia
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199202
ENTRY DATE: Entered STN: 19920315
Last Updated on STN: 19920315
Entered Medline: 19920224
ED Entered STN: 19920315
Last Updated on STN: 19920315
Entered Medline: 19920224
AB Hydrophobic affinity partitioning in an aqueous two-phase system, composed of dextran and poly(ethylene glycol), has been used to study the hydrophobic binding capacity of bovine alpha-lactalbumin. The hydrophobicity of the poly(ethylene glycol)-containing phase was adjusted by including varying amounts of fatty acids bound to the polymer via an ester linkage. The change in the logarithmic partition coefficient of the protein in such systems was used as a measure of the hydrophobic binding. This value was strongly influenced by the amount of Ca2+ present as well as the pH value. The results are discussed in terms of the exposure of hydrophobic binding sites on alpha-lactalbumin and their relation to the conformational change in this protein due to Ca(2+)-binding, chelation of Ca2+ and pH dependence.

L24 ANSWER 8 OF 16 MEDLINE on STN
ACCESSION NUMBER: 88283547 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3293985

TITLE: Effect of insulin-like growth factor I on deoxyribonucleic acid synthesis and galactopoiesis in bovine undifferentiated and lactating mammary tissue in vitro.
AUTHOR: Shamay A; Cohen N; Niwa M; Gertler A
CORPORATE SOURCE: Department of Biochemistry and Human Nutrition, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel.
SOURCE: Endocrinology, (1988 Aug) 123 (2) 804-9.
Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198808
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19880829

ED Entered STN: 19900308
Last Updated on STN: 20000303
Entered Medline: 19880829

AB We have demonstrated that insulin-like growth factor I (IGF-I), at physiological concentrations, is a potent mitogen of bovine undifferentiated mammary epithelial cells cultured in collagen in serum-free medium. Its activity is independent of insulin, although at pharmacological concentrations insulin may substitute for IGF-I. The maximal [3H]thymidine incorporation stimulated by either IGF-I or insulin was only 25-40% of that in medium supplemented with 10% fetal calf serum (FCS) only. Epidermal growth factor (EGF) exhibited low mitogenic activity which was not synergistic with IGF-I in serum-free medium. IGF-I and EGF had low synergistic activity when added separately to 10% FCS-supplemented medium. Strong synergism (100% or more) was observed, however, when both factors were added simultaneously, indicating that their maximum mitogenic effect is dependent on a simultaneous presence of other factors existing in FCS. The galactopoietic effect of IGF-I was tested in organ culture of bovine lactating mammary gland. Neither fatty acid synthesis nor alpha-lactalbumin secretion was stimulated by IGF-I, even at 2000 ng/ml. These results indicate that, at least in our in vitro system, galactopoiesis is not affected by IGF-I.

L24 ANSWER 9 OF 16 MEDLINE on STN
ACCESSION NUMBER: 87302932 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2887378
TITLE: Pituitary-induced lactation in mammary gland explants from the pregnant tammar (Macropus eugenii): a negative role for cyclic AMP.
AUTHOR: Maher F; Nicholas K R
SOURCE: Comparative biochemistry and physiology. A, Comparative physiology, (1987) 87 (4) 1107-17.
Journal code: 1276312. ISSN: 0300-9629.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198710
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19950206
Entered Medline: 19871016

Searcher : Shears 571-272-2528

ED Entered STN: 19900305
Last Updated on STN: 19950206
Entered Medline: 19871016

AB 1. alpha-Lactalbumin and casein have been isolated from tammar milk. 2. alpha-Lactalbumin was induced in mammary explants by culture with anterior pituitary. 3. Casein was induced maximally in the presence of a physiological concentration of prolactin alone. 4. Progesterone did not inhibit the prolactin-induced synthesis of casein, alpha-lactalbumin, galactosyltransferase or fatty acids. 5. Both dibutyryl cAMP and a combination of cholera toxin and IBMX did significantly inhibit the induction of casein and alpha-lactalbumin. 6. Progesterone withdrawal is not a component of the lactogenic trigger in this marsupial but cAMP may be a common intracellular signal for negative control of lactogenesis in both marsupials and eutherians.

L24 ANSWER 10 OF 16 MEDLINE on STN
ACCESSION NUMBER: 86108096 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2417826
TITLE: Inhibition of lactogenic activities of ovine prolactin and human growth hormone (hGH) by a novel form of a modified recombinant hGH.
AUTHOR: Gertler A; Shamay A; Cohen N; Ashkenazi A; Friesen H G; Levanon A; Gorecki M; Aviv H; Hadary D; Vogel T
CONTRACT NUMBER: MDO 7843-12
SOURCE: Endocrinology, (1986 Feb) 118 (2) 720-6.
Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198603
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19950206
Entered Medline: 19860305

ED Entered STN: 19900321
Last Updated on STN: 19950206
Entered Medline: 19860305

AB A recombinant analog of human GH (hGH) lacking 13 amino acids at the amino-terminus (Met14hGH) inhibited the hGH- or ovine PRL (oPRL)-stimulated proliferation of Nb2 lymphoma cells and bovine PRL-stimulated fat synthesis and alpha-lactalbumin secretion in explants from bovine lactating mammary gland. The inhibition was competitive in nature, and in Nb2 cells could be abolished by an excess of hGH or oPRL. Inhibition of oPRL-stimulated proliferation of Nb2 cells by Met14hGH could also be specifically abolished by anti-hGH monoclonal antibodies. Met14hGH had no growth-stimulating activity in Nb2 cells and was not cytotoxic. It also did not affect glucose uptake by the mammary gland explants. Met14hGH competed with [125I]hGH for binding to intact Nb2 cells, IM-9 lymphocytes, solubilized microsomal fraction from lactating bovine mammary gland, and microsomal fraction from the liver of female virgin rats, but its affinity for those receptors was 2 orders of magnitude lower than the affinity of hGH. Since Met14hGH used in most experiments contained about 25% impurities and degradation products, a small amount of it was further purified by immunoaffinity chromatography. Two purified fractions, one consisting of a single 20K protein and the other accompanied by a small amount of 25K protein, were obtained. Both fractions exhibited increased inhibition of hGH- or oPRL-stimulated

proliferation of Nb2 cells, thus indicating that the inhibitory activity results from the intact Met14hGH molecule. To the best of our knowledge, this is the first report describing the inhibition of lactogenic hormone activities by a modified hGH.

L24 ANSWER 11 OF 16 MEDLINE on STN
ACCESSION NUMBER: 86086680 MEDLINE
DOCUMENT NUMBER: PubMed ID: 4078130
TITLE: Effect of dose of bovine growth hormone on milk composition: alpha-lactalbumin, fatty acids, and mineral elements.
AUTHOR: Eppard P J; Bauman D E; Bitman J; Wood D L; Akers R M; House W A
SOURCE: Journal of dairy science, (1985 Nov) 68 (11) 3047-54. Journal code: 2985126R. ISSN: 0022-0302.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198602
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860217
ED Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860217
AB Tissue-specific effects of bovine growth hormone on lactating dairy cows were examined by analysis of milk composition. Milk samples were from 6 cows that received subcutaneous injections of 0, 5, 10, 25, 50, and 100 IU/d of growth hormone in a Latin-square design. Samples from the last 5 d of each 10-d treatment period were pooled for analyses of milk components. Concentration of alpha-lactalbumin in milk increased progressively across the treatment range up to 32% above controls (1.30 mg/nl) at the 100 IU dose. Specific alpha-lactalbumin synthesis (expressed as a percent of total milk protein) was also increased. Secretion of de novo synthesized fatty acids (short and medium chain length) in milk was increased, but response plateaued between the 50 and 100 IU/d. Secretion of preformed (long-chain) fatty acids progressively increased across the entire dose range. Thus, the percentage of long-chain fatty acids in milk increased at the highest doses of hormone. Changes in fatty acid composition of milk were apparently related to energy status; the milk response to 50 and 100 IU/d of growth hormone caused cows to be in or near negative energy balance. Exogenous growth hormone did not affect the concentration of calcium, phosphorus, sodium, iron, copper, and manganese in milk. Results are consistent with growth hormone functioning in homeorhesis to coordinate the partitioning of all nutrients to support the increased secretion of milk and milk components.

L24 ANSWER 12 OF 16 MEDLINE on STN
ACCESSION NUMBER: 81211914 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7238405
TITLE: Prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows.
AUTHOR: Akers R M; Bauman D E; Capuco A V; Goodman G T; Tucker H A
CONTRACT NUMBER: HD-09883 (NICHD)
SOURCE: Endocrinology, (1981 Jul) 109 (1) 23-30.

Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198108
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19810820
ED Entered STN: 19900316
Last Updated on STN: 19970203
Entered Medline: 19810820

L24 ANSWER 13 OF 16 MEDLINE on STN
ACCESSION NUMBER: 80117253 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7354398
TITLE: Intestinal uptake of fatty acids complexed to proteins in the chick intestine.
AUTHOR: Sklan D; Hurwitz S
SOURCE: Journal of nutrition, (1980 Feb) 110 (2) 270-4.
Journal code: 0404243. ISSN: 0022-3166.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198004
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19800417
ED Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19800417
AB Intestinal mucosal uptake of protein complexed-fatty acids was studied in ligated duodenal loops in the chick. Increasing the concentration of an albumin oleic acid-complex resulted in a linear increase in uptake of oleic acid. Varying the albumin-to-oleic-acid ratio with constant albumin concentration resulted in depressed oleic acid uptake when the ratio was below 1:3. Uptake of oleic acid complexed to albumin was increased by some 60% on addition of taurocholic acid above its critical micellar concentration. In the absence of albumin, oleic acid uptake was some 60% high from a micellar solution. Uptake of lauric acid from aqueous solution was linear with concentration until its maximum solubility was reached, whereas uptake from albumin complexes at varying lauric acid concentrations was not linear with increasing concentration. Stearic acid exhibited lowest uptake and linoleic and linolenic acid highest uptake both when complexed to albumin or from micellar solution, although albumin-complexed fatty acids were transported at about half the rate of micellar fatty acids. We concluded that some proportion of fatty acids complexed to lipophilic proteins can be absorbed in the intestine in the absence of bile acids. When oleic acid was complexed to casein, bovine serum albumin or beta-lactoglobulin at protein:oleic acid ratio of 1:10 serosal transport was 40 to 50% of mucosal uptake.

L24 ANSWER 14 OF 16 MEDLINE on STN
ACCESSION NUMBER: 75190103 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1141526
TITLE: Environmental degradation of the insect growth regulator methoprene. VIII. Boving metabolism to

natural products in mild and blood.
AUTHOR: Quistad G B; Staiger L E; Schooley D A
SOURCE: Journal of agricultural and food chemistry, (1975 Jul-Aug) 23 (4) 750-3.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197510
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19751003
ED Entered STN: 19900310
Last Updated on STN: 19900310
Entered Medline: 19751003

L24 ANSWER 15 OF 16 MEDLINE on STN
ACCESSION NUMBER: 75028539 MEDLINE
DOCUMENT NUMBER: PubMed ID: 4472930
TITLE: The mode of adsorption of proteins to aliphatic and aromatic amines coupled to cyanogen bromide-activated agarose.
AUTHOR: Jost R; Miron T; Wilchek M
SOURCE: Biochimica et biophysica acta, (1974 Aug 7) 362 (1) 75-82.
Journal code: 0217513. ISSN: 0006-3002.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197501
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19990129
Entered Medline: 19750106
ED Entered STN: 19900310
Last Updated on STN: 19990129
Entered Medline: 19750106

L24 ANSWER 16 OF 16 MEDLINE on STN
ACCESSION NUMBER: 74108257 MEDLINE
DOCUMENT NUMBER: PubMed ID: 4149947
TITLE: Metabolic adaptations during lactogenesis. Fatty acid and lactose synthesis in cow mammary tissue.
AUTHOR: Mellenberger R W; Bauman D E; Nelson D R
SOURCE: Biochemical journal, (1973 Nov) 136 (3) 741-8.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197404
ENTRY DATE: Entered STN: 19900310
Last Updated on STN: 19950206
Entered Medline: 19740425
ED Entered STN: 19900310
Last Updated on STN: 19950206
Entered Medline: 19740425

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Searcher : Shears 571-272-2528